Science Library

JUN 23 1949

45

44

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XXXV HÄFT. 2

LUND 1949, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (XXXV) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Twenty-five (25) Swedish Kronor per volume* (postage free). All back volumes are still available. The price of Vols. I—X is 10 Swedish Kronor per volume, of Vols. XI—XXIII, 15 Sw. Kr., of Vols. XXIV—XXV, 20 Sw. Kr., and of Vols. XXVI—XXXIV, 25 Sw. Kr. Thus, the price of a complete series of the periodical—Vols. I—XXXV (1920—1949)—is 585 Swedish Kronor (postage free).

ROBERT LARSSON
Editor of »Hereditas»
3 Adelgatan, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *ARNE MÜNTZING*
PROFESSOR, FIL. DR. *HERIBERT NILSSON*
PROFESSOR, FIL. DR. *GERT BONNIER*
PROFESSOR, MED. DR. *ERIK ESSEN-MÖLLER*

STUDIES ON BIRCH SPECIES HYBRIDS

I. BETULA VERRUCOSA × B. JAPONICA, B. VERRUCOSA × B. PAPYRIFERA AND B. PUBESCENS × B. PAPYRIFERA

BY *HELGE JOHNSSON*

FOREST TREE BREEDING INSTITUTE, EKEBO, KÄLLSTORP, SWEDEN

SOME years ago the author published a first report on a number of experiments which are being conducted with species hybrids within the genus *Betula* at the Swedish Forest Tree Breeding Institute (JOHNSSON, 1945). Since then these experiments have been extended to new hybrid combinations concomitantly as the hybrids produced earlier have been under continuous observations. In the meantime the older material has attained flowering age. This applies especially to the hybrids produced in 1941: *Betula verrucosa* × *B. japonica, B. verrucosa* × *B. papyrifera* and *B. pubescens* × *B. papyrifera*, which in the spring of 1948 flowered generally and abundantly. As a result of this it was possible to study the floral morphology, course of meiosis and fertility conditions, the results of which are submitted below.

VEGETATIVE DEVELOPMENT.

The three hybrids are grown together with an individual offspring of each species, *B. verrucosa*, *B. pubescens* and *B. papyrifera*, on formerly tilled land with a fairly clayey and highly calcareous soil. Planting was carried out as a block test according to FISHER with three repetitions. The plots comprise two rows with originally 50 trees in each row. For different reasons a few trees failed to grow, and the number of trees per plot in the spring of 1948 was 40—48. The original distance between trees was 1,2 × 1,2 m. Planting was effected here when in the spring of 1943 the hybrids were one year old. As early as the year after planting the hybrids showed a considerably better growth in height than the pure species *B. verrucosa* and *B. pubescens* (JOHNSSON, 1945). This superiority of the hybrids in respect of growth has become increasingly evident. In the autumn of 1948, when the trial was seven years old, measurements were made and gave the results listed in Table

TABLE 1. *Measurements of 7 years old hybrids and pure species.*

Hybrid or species	Height m.	Diam. cm.	Cubic volume dm.³	Number of trees har	Cubic volume m³/har
1. *B. verrucosa* × *papyrifera*	4,68	2,83	3,56	6,574	23,40 ± 2,24
2. *B. pubescens* × *papyrifera*	4,75	2,90	3,55	5,278	18,75 ± 1,79
3. *B. verrucosa* × *japonica*	4,98	3,23	5,00	6,250	31,25 ± 2,13
4. *B. papyrifera*	5,28	3,27	4,88	5,417	26,45 ± 1,84
5. *B. pubescens*	4,59	2,44	2,56	5,324	13,65 ± 1,81
6. *B. verrucosa*	4,25	2,33	2,15	6,574	14,16 ± 2,24
Mean errors	0,17	0,14	0,34		

1. The diameter was measured at half the height of the trees and the volume was estimated as that of a cylinder having the same diameter. Analyses of the variance give the following significances:

Height differences in cm.

	1.	2.	3.	4.	5.
6.	43*	50**	73***	103***	34*
5.	9	16	39*	69**	
4.	60**	53**	30*		
3.	30*	23			
2.	7				

Diameter differences in mm.

	1.	2.	3.	4.	5.
6.	5,0***	5,7***	9,0***	9,4**	0,4
5.	3,9**	4,6**	7,9***	8,3***	
4.	4,4**	3,7*	0,4		
3.	4,0**	3,3*			
2.	0,7				

Volume differences per tree in dm³.

	1.	2.	3.	4.	5.
6.	1,41**	1,40**	2,85***	2,73***	0,41
5.	1,00**	0,99**	2,44***	2,32***	
4.	1,32**	1,33**	0,12		
3.	1,44***	1,45***			
2.	0,01				

The numbers 1—6 refer to the order in which the varieties are listed in Table 1. Thus, the six varieties are distributed over three groups with significantly different rates of growth. First in this respect come *B. verrucosa* × *japonica* and *B. papyrifera*, next come the two *papyrifera* hybrids, and last *B. verrucosa* and *B. pubescens*. All the hybrids accordingly possess a powerful heterosis in comparison with the two

species *B. verrucosa* and *B. pubescens*, but *B. papyrifera* holds its position well in competition with the hybrids. In this comparison, however, account has to be taken of a couple of disturbing elements. It seems to be a general phenomenon that a moderate shift to the north *in et per se* increases the growth of woody plants. The exact provenance of the *papyrifera* material employed in the experiment is not known — the mother is a tree cultivated in the Botanical Garden of Lund — but in all probability it has a considerably more southern origin than the latitude of the district in which it is at present growing, which is about 56° N. Lat. Further, the *papyrifera* family would seem to have been favoured to some extent by the fact that at the laying-out of the trial the plants were two years old, while the hybrid material was one year old. Undoubtedly the results of the trial justify the conclusion that the hybrids possess a manifest heterosis that has evoked an increase in growth which has given more than double as large a volumetric growth for *B. verrucosa* × *japonica* at 7 years of age than for pure *B. verrucosa*. For *B. verrucosa* × *papyrifera* the increase is 66 % compared with *B. verrucosa*, and for *B. pubescens* × *papyrifera* 39 % compared with pure *B. pubescens*. Quite naturally, it is impossible to say whether this heterosis effect will also manifest itself during the continued development of the trees. So far as can be judged, however, these hybrids represent a valuable cultivating material for reforestation. In mode of growth and quality of branching they also answer highly placed demands. On the other hand, in spite of its good rate of growth the *B. papyrifera* type included in the trial is of little value on account of its crooked stem and its very thick branches.

MORPHOLOGICAL CHARACTERS.

Those individuals of *B. verrucosa* and *B. pubescens* which were used as hybrid parents are in their principal features typical of their respective species. In form of branching the *B. verrucosa* mother represents an extreme *pendula* type, probably of Danish descent. The *B. pubescens* mother is of South-Swedish origin. The paternal tree of *B. papyrifera* is grown in the Lund Botanical Garden under the name of *B. papyrifera* var. *occidentalis*. That the tree belongs to the collective species *B. papyrifera* MARSH is beyond all doubt, but it is not possible to decide whether or not it belongs to var. *occidentalis* (see below). The *papyrifera* family included in the progeny trials has the same tree as mother. At eight years of age all the progeny now possess a very dark bark varying

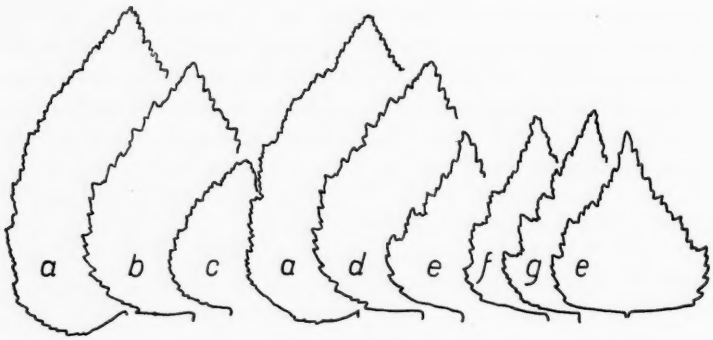


Fig. 1. Leaf contours: *a* = *B. papyrifera*, *b* = *B. pubescens* × *papyrifera*, *c* = *B. pubescens*, *d* = *B. verrucosa* × *papyrifera*, *e* = *B. verrucosa*, *f* = *B. japonica*, *g* = *B. verrucosa* × *japonica*.

in shade from reddish-brown to leaden-grey. As a rule the annual shoots are very warty with thinly set, stiff hairs round the axils. Only 7 of the remaining 115 trees, or 6,10 %, are hairy to any noticeable extent. The leaves (Fig. 1) as well as catkin-scales and fruits (Figs. 2 and 4) fall well within the variation-range of *B. papyrifera*. The father tree

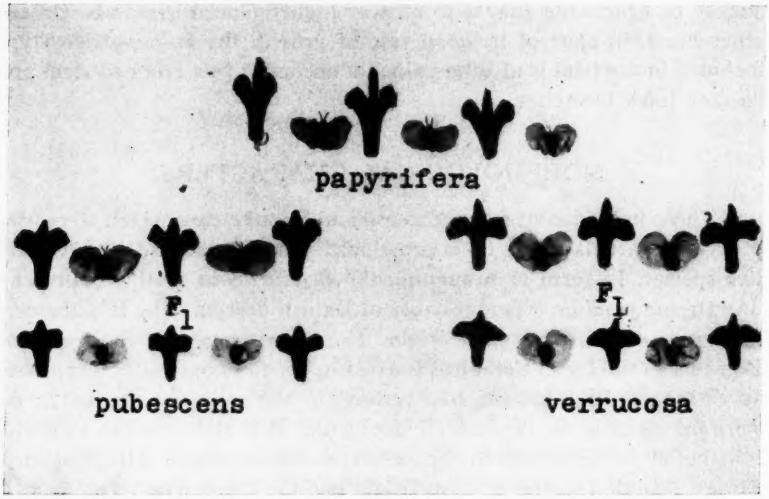


Fig. 2. Catkin-scales and fruits of *papyrifera* hybrids and their parental species.

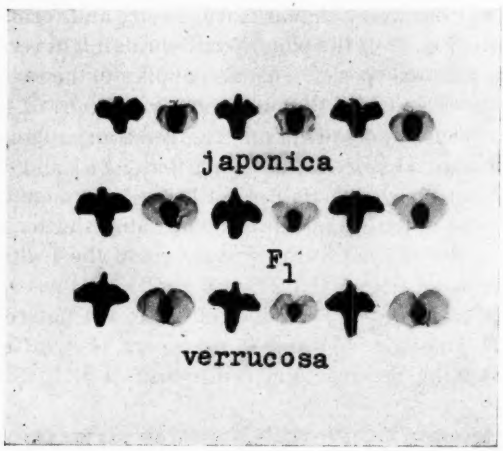


Fig. 3. Catkin-scales and fruits of *B. verrucosa* × *japonica* and its parents.

of *B. japonica* is grown in the Bergian Gardens of Stockholm. In every way the tree can be regarded as a good representative of *B. japonica* SIEBOLD (see Figs. 1, 3 and 4). All the hybrids are intermediate. In Fig. 1 the leaf-form of the hybrids compared with that of the parental species is illustrated. The type of *B. papyrifera* used has leaves that are several times larger (Fig. 1 *a*) than the other species dealt with here (Fig. 1 *c*, *e*, *f*). The size of the leaves in the hybrids, *B. pubescens* × *papyrifera* (1 *b*) and *B. verrucosa* × *papyrifera* (1 *d*), is perfectly intermediate. Both the species *B. verrucosa* (1 *e*) and *B. japonica* (1 *f*) and

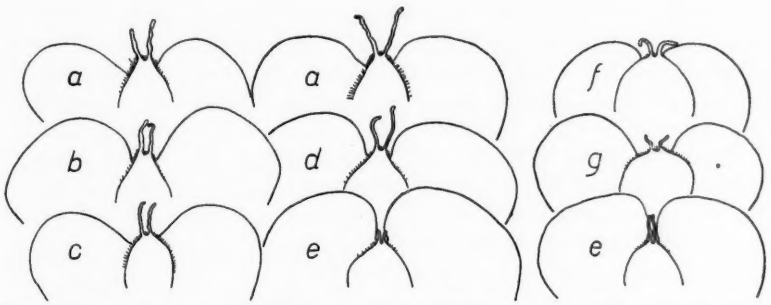


Fig. 4. Fruits of: *a* = *B. papyrifera*, *b* = *B. pubescens* × *papyrifera*, *c* = *B. pubescens*, *d* = *B. verrucosa* × *papyrifera*, *e* = *B. verrucosa*, *f* = *B. japonica*, *g* = *B. verrucosa* × *japonica*. — × 5.

their hybrid (1 *g*) have very similar leaves in size and form. The catkin-scales and fruits (Fig. 2) of the *papyrifera* hybrids might very well belong to either of the parental species. This also applies to the hybrid *B. verrucosa* × *japonica* (Fig. 3). In Fig. 4 the upper portions of the fruits are represented under 5 × magnification. The persisting stigmata are taxonomically valuable characters. *B. papyrifera* (4 *a*) and *B. pubescens* (4 *c*) agree in that their stigmata project beyond the edge of the wings; in *B. verrucosa* (4 *e*) the stigmas are considerably shorter. Still, the relative length of the stigmas varies greatly [both the fruits (*a*) are derived from the same tree, and the same applies to the two fruits (*e*)]. *B. verrucosa* × *papyrifera* approaches closer to the paternal species in this respect. *B. japonica* (4 *f*) and *B. verrucosa* (4 *e*) differ from each other in a striking manner, and the hybrid (4 *g*) is illusorily intermediate.

In *B. verrucosa* × *papyrifera* the annual shoots are perceptibly warty throughout. Only 13 of 145 trees, or 8,97 %, are also distinctly hairy. The annual shoots in *B. pubescens* × *papyrifera* may be warty to some extent but are mostly bare or more or less hairy. Of 111 trees, 61 or 54,96 % are distinctly hairy.

CHROMOSOME NUMBERS.

The chromosome number of *B. verrucosa* is $2n = 28$ according to counts made by several workers (HELMS and JØRGENSEN, 1927; WOODWORTH, 1931; WETTSTEIN and PROPACH, 1939; JOHNSSON, 1941, 1944, 1945). Exceptionally occurring autotriploids constitute the single departure from this number (JOHNSSON, 1944). So far as is known, this also applies to the chromosome number of *B. japonica* (WOODWORTH, 1931; JOHNSSON, 1945). For *B. pubescens* only the number $2n = 56$ has been given (HELMS and JØRGENSEN, 1927; WOODWORTH, 1931; WETTSTEIN and PROPACH, 1939; JOHNSSON, 1941, 1944, 1945). For *B. papyrifera* the following numbers have been found by WOODWORTH (1931).

B. papyrifera var. *cordifolia*	$2n = 56$
B. papyrifera var. *subcordata*	$2n = 56$
B. papyrifera	$2n = 70$
B. papyrifera var. *kenaica*	$2n = 70$
B. papyrifera var. *occidentalis*	$2n = 84$

If these numbers are universally applicable, chromosome counts should afford a reliable means of determining var. *occidentalis*. This

TABLE 2. *Chromosome numbers of B. papyrifera plants from different origin.*

Origin	Number of mother trees	Chromosome numbers of daughter plants															Number of daughter plants
		70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	
British Columbia	5	1			4	4	3	2	1		1			1	4	5	26
Washington ...	1											2					2
Minnesota ...	7	3	2	1			2		2			2		2	3	14	31
Mass. and N. H.	6	1			1	1	2		1			1		2	1	4	14
N. B. and P. Q.	2				1		3							1	1	2	8
Ontario	2				2		1									5	8
Total	23	5	2	1	8	5	11	2	4	0	1	5	0	6	9	30	89

variety is considered by REHDER (1940) to occur in British Columbia, Washington and up to Montana. The number $2n = 84$, however, is of far commoner occurrence within *B. papyrifera*. At chromosome counts of 89 daughter-plants from 23 maternal trees growing in different localities in North America (the seeds of these plants were gathered on the sites of growth) the author obtained the results submitted in Table 2. On account of the high chromosome numbers and of the frequently poor quality of the prepared specimens the counts are subject to a certain degree of uncertainty, which amounts to at least ± 2 chromosomes but in no circumstances exceeds ± 5 chromosomes. Without doubt the counts more often give a chromosome or so too few than too many. Thus, the somatic number $2n = 84$ occurs within *B. papyrifera* not only in the West but also in the Middle West, Ontario, New England as well as New Brunswick and Quebec. If 56-chromosomal forms also occur generally, individuals with 70 chromosomes may arise through hybridization between 56- and 84-chromosomal types. Should hybrids between 70- and 84-chromosomal types be formed, the product would be 77-chromosomal individuals. The distribution of the different chromosome numbers in Table 2 indicates an accumulation round $2n = 75$, which may reflect a hybridization of this kind. It accordingly appears as though the conditions of polyploidy within *B. papyrifera* are considerably more complicated than is suggested by WOODWORTH's investigation.

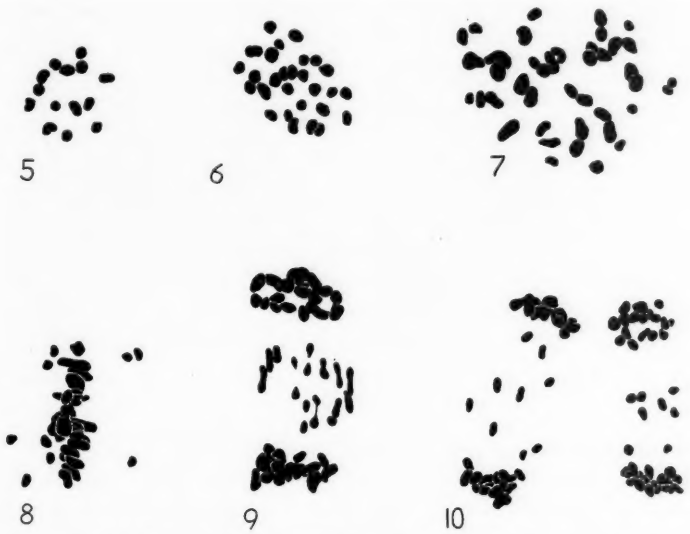
The material studied in the present work has the following chromosome numbers:

B. verrucosa $2n = 28$, typical of the species;
B. japonica $2n = 28$, » » » »
B. pubescens $2n = 56$, » » » »
B. papyrifera $2n = 84$, typical of a large portion of the species;
B. verrucosa × *japonica* $2n = 28$
B. verrucosa × *papyrifera* $2n = 56$
B. pubescens × *papyrifera* $2n = 70$

COURSE OF MEIOSIS.

B. verrucosa × *japonica* possesses a normal meiosis with 14 bivalents (Fig. 5). Only very rarely can two univalents be observed. Such a very weak asyndesis may also occur in pure *B. verrucosa*. The meiosis has been studied in 13 F_1 individuals, and hundreds of anaphases from the side have been observed. In no case, however, has any symptom of inversion been found. The second division is as regular as the first (Fig. 11). Hence the genomes in *B. verrucosa* and *B. japonica* seem to be identical as regards meiosis-affecting structure. *B. verrucosa* × *papyrifera* has 14 chromosomes from the maternal species and 42 from the paternal. The I-M appears to be free from any disturbances and probably always shows 28 bivalents (Fig. 6). Meiosis has been studied in 12 F_1 trees with the same result. Two univalents make their appearance in very low frequency. It is possible, though not probable, that multivalents occur. The chromosomes are relatively small and numerous, at the same time as a certain amount of stickiness is noticeable (Fig. 11). This means that it cannot be decided with certainty whether two bivalents in contact with each other are joined by chiasmata or not. The frequent fusion of the chromosomes into larger or smaller aggregations might also be conceived as caused by secondary association. However, this sticky-like aggregation also makes its appearance in the diploid material, as will be seen from the microphotographed II-M plate in Fig. 11. No doubt can prevail that the meiosis in the F_1 *B. verrucosa* × *papyrifera* displays 28 bivalents with great regularity. Two of the *papyrifera* genomes must accordingly pair autosyndetically and the third with the *verrucosa* genome, or in other words a *papyrifera* genome is structurally identical with the *verrucosa* genome.

Autosyndesis in a birch hybrid has been earlier reported by HELMS and JØRGENSEN (1927) for *B. verrucosa* × *pubescens*. This report is erroneous, however. Morphologically, the spontaneous 42-chromosomal tree investigated by them agreed completely with *B. verrucosa* and must



Figs. 5—10. Fig. 5. *B. verrucosa* × *japonica*, I-M, polar view, 14_{II}. — Fig. 6. *B. verrucosa* × *papyrifera*, I-M, polar view, 28_{II}. — Figs. 7—10. *B. pubescens* × *papyrifera*, Fig. 7. I-M, polar view, ± 41 separate bodies. — Fig. 8, I-M, side view, showing numerous univalents. — Fig. 9. I-A, side view, with lagging, dividing univalents. — Fig. 10. II-A, side view, with lagging univalents. — × 3500.

be an autotriploid, since the F_1 *B. verrucosa* × *pubescens* is quite intermediate (JOHNSSON, 1944). In all probability this autotriploid did not have a regular meiosis with 21 bivalents, but had trivalents as well as bivalents and univalents, the sum of which may have often been 21. It is also very difficult — not to say impossible — to distinguish with certainty in birch between trivalents, bivalents and univalents in the I-M in polar view (JOHNSSON, 1944). A drawing of a I-A in side view reproduced by HELMS and JØRGENSEN distinctly shows three univalents in division.

B. pubescens × *papyrifera*, which possesses 70 chromosomes, 3 × 14 from *B. papyrifera* and 2 × 14 from *B. pubescens*, shows an entirely divergent course of meiosis with considerable disturbances. Meiosis cannot be exactly analysed here, either, owing to the above-mentioned difficulties. Fig. 7 shows the I-M in polar view with ± 41 separate bodies of different size and often in contact with one another. In Fig. 8 the I-M is seen in side view with 10 distinctly discernible univalents outside the metaphase plate. A larger or smaller number of

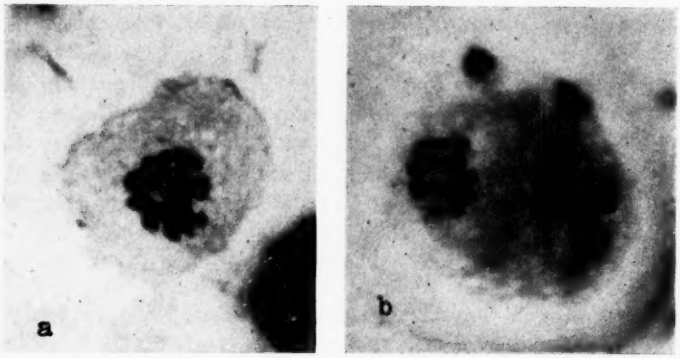


Fig. 11. *a* = *B. verrucosa* × *papyrifera*, I-M. — *b* = *B. verrucosa* × *japonica*, II-M. — × 2900.

univalents can always be observed, mostly 5—10. Actually, the frequency of univalents is higher, since it may be taken that there are always some univalents concealed by the metaphase plate. Multivalents have not been observed. The I-A in side view invariably shows a large number of univalents lagging behind at different stages of division, as in Fig. 9. No secondary equatorial plate is formed by the univalents. In the II-A a large number of lagging univalents invariably make their appearance, as will be seen from Fig. 10. It seems justifiable to assume that the two *papyrifera* genomes that pair autosyndetically in *B. verrucosa* × *papyrifera* also do so in this hybrid and that the remaining *papyrifera* genome pairs with one of the *pubescens* genomes while the other *pubescens* genome appears for the most part as univalents. Possibly one or two chromosomes from the odd genomes become embodied in trivalents. A certain amount of intragenomic pairing may also occur. The *pubescens* genomes seem to stand in the same relation to one of the *papyrifera* genomes as they do to the *verrucosa* genome in the hybrid *B. verrucosa* × *pubescens* (see JOHNSSON, 1944). In view of the fact that in the hybrid *B. verrucosa* × *papyrifera* the *verrucosa* genome has been found to be homologous to the *verrucosa* genome, this is also to be expected. The course of meiosis was in complete agreement in all the 9 F_1 individuals examined.

FERTILITY CONDITIONS.

In Sweden during 1948 birch had in general an extremely copious flowering and seed-production. With due account taken to its young

age this was also the case with the trial plantation dealt with in this paper. All the hybrids flowered more richly than the pure species. An analysis of the male fertility is summarized in Table 3. Of *B. verrucosa* × *japonica*, 85 F_1 individuals were examined. Fourteen of these were diplontically sterile, since the anthers did not open in spite of their pollen contents appearing to be normal. The remaining 71 trees spread their pollen unhindered, and 52 (73 %) of them possessed as perfect pollen as a pure species. There was a varying though not specially high incidence of empty pollen-grains in 19 trees. On an average the percentage of good pollen was 94,25 % for this hybrid. Of *B. verrucosa* × *papyrifera* 58 trees could be examined with reference to their male fertility. Of these, 28 or almost one-half were diplontically sterile. The diplontic sterility was here far more pronounced than in *B. verrucosa* × *japonica*. The flowers of the male-sterile trees were very abnormal with rudimentary anthers in which archespores were never differentiated. The normally male-flowering 30 siblings often possessed a perfect pollen or a pollen having a low incidence of empty grains. Only a couple of trees showed any substantial deterioration in pollen quality. On an average the rate of good pollen was 90,29 %. The hybrid *B. pubescens* × *papyrifera* presented a pollen picture that was entirely divergent from the two preceding ones. It exhibited no diplontic sterility. Its pollen quality was much reduced, showing an average of only 67,57 % good grains. There was a great variation ranging from 5—10 % up to 90—95 % good pollen. Thus, the nature of the pollen in the three hybrids was found to be a true reflection of the meiotic course. Both the F_1 combinations *B. verrucosa* × *japonica* and *B. verrucosa* × *papyrifera* had regular meiosis and a pollen that was quite satisfactory or only slightly deteriorated. The low frequency of poor

TABLE 3. ♂-*fertility* (*D* = *diplontic sterile plants*).

Hybrids	D	Numbers of plants with respective percentages good pollen grains																			Means	n
		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95		
B. verrucosa × *japonica*	14													1	1	3	2	2	4	6	52	94,25	71
B. verrucosa × *papyrifera*	28											2			1	1		3	3	3	17	90,29	30
B. pubescens × *papyrifera*	0		1	1		1	1	2	2	3	7	5	8	5	11		7	10	8	8		67,57	82

TABLE 4. *Percentages germinability of crosses hybrids × pure species.*

♂ \ ♀		*B. verrucosa*		*B. pubescens*	
		No. 1	No. 2	No. 1	No. 2
B. verrucosa × *japonica*	No. 8	46,5	44,5		
» »	No. 13			14,0	
» »	No. 20				1,0
B. verrucosa × *papyrifera*	No. 20			39,0	6,3
B. pubescens × *papyrifera*	No. 126		18,5		
» »	No. 127	27,3	16,0		
» »	No. 129			30,0	

pollen here can quite certainly be assigned to genic causes, as may also the diplontic sterility. *B. pubescens* × *papyrifera* had a much disturbed meiosis with a high frequency of univalents and, corresponding to this, a powerful falling off in the pollen quality owing to unbalanced haplolethal chromosome combinations.

The functioning ability of the hybrid pollen has been subjected to a preliminary test by using it for pollinating trees belonging to the two species *B. verrucosa* and *B. pubescens*. The percentage germination of the resulting seed tested on JACOBSEN's apparatus is recorded in Table 4. Tested by the same method, variety crosses within *B. verrucosa* and *B. pubescens* showed a germination that as a rule exceeded 50 % but was as low as 20 % or less in a case here and there. The results recorded in Table 4 show that the pollen of all three hybrids has so good a functioning capacity, at any rate in some combinations, that pollination with adequate quantities can produce a seed of relatively high germinability. Tests were also made of the seed of the hybrids after open pollination, 3 × 100 fruits from every examined tree being analysed and the frequency of »fruits with seed» determined. Birch fruits develop irrespective of whether pollination takes place or whether the ovule has aborted at an early stage. In variety crosses without complications the actual germinability is very close to the maximum. A comparison of the female fertility expressed in this way for the three hybrids is given in Table 5. The 88 tested F_1 individuals belonging to *B. verrucosa* × *japonica* had an average maximum germinability of 53,3 %, which in itself is as high as for pure *B. verrucosa*. However, the dispersion was great, though hardly greater than for pure *B. verrucosa*. Both the *papyrifera* hybrids, on the other hand, had a considerably reduced germinability. For *B. verrucosa* × *papyrifera*, of which com-

bination 52 individuals were tested, the germinability averaged only 7,3 % and for more than one-half of the trees did not exceed 5 %. This contrasts sharply with the regular meiosis, and also with the high percentage rate of good pollen. However, almost one-half of the individuals were diplontically male sterile. There may also be diplontic sterility on the female side. In an autosyndetic hybrid, as this is, a low germinability is not of course surprising. Despite the structural homology considerable genetic differences are to be expected between the genomes, and hence unbalanced and lethal gene combinations are liable to arise at the recombination of chromosomes taking place during the meiosis of the hybrid. A still more reduced germinability was shown by *B. pubescens* × *papyrifera*. Of 91 trees tested, only one had more than 5 % germinable seeds. In most of the others no germinable seed was found at the analysis. The principal cause of this sterility is doubtless to be sought in the deranged reduction division with its consequent gamete- and zygote-lethality.

The possibility and suitability of utilizing in forestry the heterosis effect in a species hybrid by cultivating a certain hybrid on a large scale is dependent on a number of circumstances. If the kind of tree in question is propagated vegetatively in current practice or can be multiplied by that method at reasonable cost, a single F_1 individual in possession of good technical properties will of course afford adequate starting-material. This is the case with poplar. If, on the other hand, the tree-species cannot be propagated vegetatively in practice, hybridogenous seed must admit of being produced in sufficient quantities at reasonable cost. Vegetative propagation is certainly possible in birch by grafts as well as cuttings, but this procedure is doubtless too expensive.

TABLE 5. ♀-*fertility*.

Hybrids	Numbers of plants with respective percentages fruits containing seeds																			Means	n
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95		
B. verrucosa × *japonica*	3		1	3	2	4	5	3	9	3	3	12	8	12	6	11	2	1		53,30	88
B. verrucosa × *papyrifera*	31	7	6	4	1	1	1				1									7,28	52
B. pubescens × *papyrifera*	90	1																		0,15	91

Recourse must therefore be had to producing valuable hybrids by seed. Two alternatives are then conceivable, to use in practice either the F_1 or the F_2 (and later generations). If the F_1 is employed, the two parental species must be so interfertile that a seed having sufficient germinability is obtained for the mass production of the F_1. For the hybrids under discussion here the germinating capacities of the species-crosses (JOHNSSON, 1945) were: *B. verrucosa* × *japonica* = 53,2 %, *B. verrucosa* × *papyrifera* = 8,8 %, *B. pubescens* × *papyrifera* = 1,4 %. The germinability for the first-mentioned cross is as good as for pure *B. verrucosa* in general. *B. verrucosa* × *papyrifera* can also be considered to possess sufficient fertility in practice. *B. pubescens* × *papyrifera*, on the other hand, yields a seed of too low germinating power. Interfertility between two species must, however, depend in some degree on the individual constitution of the parental individuals used in each special case. In particular must this be so in the case of races which have different chromosome numbers but are of one and the same species, as certainly occurs in *B. papyrifera*. If the F_2 is to be used, the condition of primary importance is that the F_1 possesses sufficient fertility for a large F_2 to be raised. In the case of the seed production in question here of the F_1 hybrids after open pollination, the seed-setting mainly took place after spontaneous pollination within each hybrid separately. The three hybrids have flowering times that are fairly well separated from each other. *B. verrucosa* × *japonica* is 1—2 weeks earlier than the *papyrifera* hybrids, and of these *B. verrucosa* × *papyrifera* flowers somewhat earlier than the other. Moreover, as the hybrids flowered considerably more copiously than the pure species contained in the same plantation, there was probably no great incrossing from such. The values obtained for the germinability of the seed may therefore be regarded as good approximate values for the intrafertility of the F_1 hybrids. It is, then, forthwith clear that by selection of individuals with high germinability in *B. verrucosa* × *japonica* as well as in *B. verrucosa* × *papyrifera* the F_2 can easily be produced in large numbers.

In *B. pubescens* × *papyrifera*, however, this is probably not the case. Use of the F_2 would, of course, presuppose that the heterosis effect also remains in this generation. In species crosses with a fertile F_1 in general, however, the F_2 shows strong segregation and on an average is inferior to the F_1 as well as to the parental species. Still, in forestry practice only a certain portion of the seedling stand is raised for utility purposes, and therefore the mean values of the population are not decisive if only a sufficiently large fraction of the F_2 is as good as the

F_1. An F_2 population can doubtless be considered of use if about one-half of the individuals are equal to the F_1. However, this requirement would seem to be extremely seldom fulfilled and as a rule the F_2 cannot be employed. Despite this, the fertility of the F_1 is of great — though negative — interest.

If the practice within the area in which the hybrid is to be grown is based to a rather wide extent on natural regeneration — as in Sweden — it is undoubtedly most advantageous if the F_1 is entirely sterile. A culture of fast-growing, fertile F_1 hybrids is otherwise liable to be succeeded in the future by an inferior F_2 population that has arisen through natural reproduction. For the hybrid *B. pubescens* × *papyrifera* there is no such risk, for *B. verrucosa* × *papyrifera* the risk is small, while for *B. verrucosa* × *japonica* the risk must be regarded as great.

Fulfilment of one of the two following conditions is nevertheless conceivable: (1) either that a sufficiently large part of the F_2 is of quite satisfactory character or (2) that the whole F_2 population has so low a vitality that it is unable to maintain itself in competition with other reproductions.

Hence, to secure full clarity as to the value of an F_1 hybrid as a forest tree, the growing capacity of the F_2 must also be studied, as the recommendations for the treatment of an F_1 cultivation must depend on the results of this study. If, on the other hand, the hybrid is to be grown within an area in which natural regeneration is not applied — as in large parts of Central Europe —, the fertility of the F_1 is evidently of no consequence. Still, a pronounced diplontic sterility with rudimentary flowers or an entire failure of flowering would be preferable even in such cases, for during a year of abundant flowering efflorescence and seed-production requisitions a considerable portion of the dry matter produced during the year, with the result that the growth of wood — the utility product — is reduced.

DISCUSSION.

The demarcation of species within the genus *Betula* is undoubtedly a difficult matter owing to a wide and transgressive variation. It is just as certain that birch taxonomy has been rendered still more difficult by a number of investigators who have dealt with the genus. Numerous species, subspecies, varieties and subvarieties have been set up on a frightfully inadequate foundation and without knowledge of the geographical conditions of distribution.

For instance, Scandinavia's arborescent birches — the Linnean *B. alba* — have been the object of the most hair-raising taxonomic attempts. KINDBERG (1909) set up 22 »species» of equal rank. GUNNARSSON (1925) contented himself with five »species» but regarded practically every tree as a hybrid between two or more species. Further taxonomic units, which as a rule are denoted as varieties have been created by other authors, among them most recently by LINDQUIST (1947).

It has, however, proved that in Scandinavia we have two well distinguished intrafertile and intersterile spheres of variation within the tall birches, the 28-chromosomal *B. verrucosa* and the 56-chromosomal *B. pubescens* (JOHNSSON, 1945; for synonyms, see FERNALD, 1945). Whether the fjeld (or mountain) type of *B. pubescens* — *B. tortuosa* LEDEB. — is to be interpreted as a separate species, a subspecies or a variety, or as contained in the variation-sphere of *B. pubescens*, is primarily a matter of taste. The same may be said to be the case with the *B. verrucosa* varieties var. *lapponica* and var. *saxatilis* set up by LINDQUIST (1947). According to LINDQUIST, var. *saxatilis* is southerly and var. *lapponica* northerly. Between them he considers that there is a broad transitional zone.

Within Scandinavia — and certainly also in Asia and North America — the gene frequencies of the birch populations show a continuous change that runs parallel to climatic changes, *i. e.* a pure clinal variation. The climatic factors of selection have exercised a direct pressure on physiological properties, and this has frequently also manifested itself in morphological changes. In such a continuous variation it is obviously always possible to distinguish extreme types connected by a broad zone of transition. Whether such a procedure is justified or not, may be discussed.

The two principal species *B. pubescens* and *B. verrucosa* extend far into Asia, and within these enormous areas innumerable varieties can undoubtedly be distinguished. In fact, several have been described and given names. In a work of 1902 by FERNALD »The relationships of some American and Old World Birches» that author mentions the great agreement between Eurasiatic and North-American forms of *B. alba*. In broad outline FERNALD then arrived at the following result as regards the *B. verrucosa* range of forms: *B. kenaica* EVANS, North America = *B. alaskana* SARG. (*B. neo-alaskana* SARG.), North America = *B. alba* subsp. *verrucosa δ resinifera* REGEL, North America and Asia = *B. pendula* ROTH (*B. verrucosa* EHRH.) var. *japonica* SIEBOLD, Asia; and as regards the *B. pubescens* sphere of forms FERNALD gave: *B. papyrifera*

MARSH, North America = *B. alba* L., *B. pubescens* EHRH., Eurasia = *B. occidentalis* HOOK, North America (see FERNALD, 1902, for complete discussion).

Later (1945) FERNALD repudiated this classification of his of the year 1902. He says: »That sophomoric study, based on complete lack of understanding, well illustrates how an over-conservative treatment may be as far afield as are those which split beyond the normal divergencies of Nature». Quite recently, however, LINDQUIST (1947) re-advanced FERNALD's views of the year 1902 as regards the *B. verrucosa* form-circle in almost exactly the same way.

The cytological and experimental data hitherto gathered are still too limited to allow of a more comprehensive treatment of the birch genus as a whole. Still, those already at our disposal give useful hints. LINDQUIST's (1947) statement that the chromosome number 56 »is found to be prevalent among most of the species belonging to the Alba» is incorrect. In the first place, all *verrucosa* types whose chromosome numbers are known have $2n = 28$. The hybrid *B. verrucosa* × *japonica* treated here was found to possess a regular meiosis with 14 bivalents. This substantiates the view held by FERNALD (1902) and earlier botanists of the existence of a close kinship between the European *B. verrucosa* and the Asiatic *verrucosa*-like type.

WOODWORTH (1931) published a series of chromosome numbers for *B. papyrifera* that show an elegant parallelism between systematic division and chromosome number (see above). The material dealt with here, however, shows that the chromosome number 84 is not confined to v. *occidentalis* but occurs, in any case within the entire southern part of the *B. papyrifera* area, together with different numbers between 70 and 84. FERNALD (1945), however, has altered the name v. *occidentalis* HOOK, which was originally described from the lower Fraser River, B. C., and Vancouver Island and had always been interpreted as a distinct Pacific form that did not extend further eastwards than to Montana (see, *e. g.*, HULTÉN, 1944), to v. *commutata* REGEL comb. nov. In this he includes all *papyrifera* birches with dark and unpeeling bark and mentions habitats in Massachusetts also. However, within such characteristically white-stemmed species as *B. verrucosa* and *B. pubescens* there also occur very dark-barked individuals as extreme types in a continuous variation. These »gray birches» are in fact considered — rightly or wrongly — to be of specially high technical value. In all probability the dark-barked *B. papyrifera* trees in Massachusetts are just such extreme types and probably have nothing to do with either v.

occidentalis HOOK or the chromosome number 84. Undoubtedly at least the major portion of *B. papyrifera* forms a variation sphere well separated from *B. pubescens* and characterized by higher chromosome numbers. Judging from everything, the polymorphy of the species is at least partially explicable by the fluctuation of the chromosome number between 70 and 84. It would be particularly interesting if a re-investigation of a large spontaneous material of the more northerly varieties v. *subcordata* and v. *cordifolia* could confirm that they are actually characterized by the $2n = 56$ number like the Eurasiatic *B. pubescens*.

The $2n=56$ hybrid *B. verrucosa* $\times$ *papyrifera* treated here possesses a regular meiosis with 28 bivalents. Hence two *papyrifera* genomes must be sufficiently homologous for autosyndetic pairing and the third be homologous to the genome of *B. verrucosa*. By genomic symbols, therefore, the hexaploid *B. papyrifera* could be denoted as $\frac{P_1P_2V}{P_1P_2V}$, *i. e.* as a synthesis of a *verrucosa*-like species and an autotetraploid of other origin. Maybe this autotetraploid is to be sought in one of WOODWORTH's 56-chromosomal varieties.

Two important questions in this connection are: (1) what relations exist between the 56-chromosome *papyrifera* types and *B. pubescens*, (2) by what paths has the *verrucosa* genome been introduced into *B. papyrifera*?

Probably *B. pubescens* is not an autotetraploid but an allopolyploid, made up of a *verrucosa*-like type and another diploid species. Indicative of this is the course of meiosis in *B. verrucosa* $\times$ *pubescens* as well as in *B. pubescens* $\times$ *papyrifera*, if the latter is viewed in relation to *B. verrucosa* $\times$ *papyrifera*. The *verrucosa* genome may of course have been introduced into *B. papyrifera* directly through a diploid, *verrucosa*-like form or indirectly through an allotetraploid having a *verrucosa* genome (a *B. pubescens* type?). The first-mentioned possibility does not appear improbable. The most easterly *verrucosa*-like form occurs in Kamchatka, *B. platyphylla* SUKATCHEV, syn. *B. japonica* SIEBOLD (HULTÉN, 1928). In Alaska there are to be found two *verrucosa*-like types, *B. kenaica* EVANS and *B. resinifera* BRITTON, syn. *B. neoalaskana* SARG. *B. kenaica* seems to be a species with a limited spread, being confined to the Kenaica peninsula and the base of the Alaska peninsula, while *B. resinifera* occurs from Alaska through Yukon to Saskatchewan (HULTÉN, 1944). These *verrucosa*-like birches in Alaska apparently bear a strong resemblance to the East-Asiatic *verrucosa*-like forms. Referring to *B. platyphylla* in Kamchatka HULTÉN (1928) says:

»I have seen specimens from Alaska under the name of *B. alaskana* and *B. kenaica*, that are closely related to our form. The birch of northern Japan also belongs to our species». And in his Alaska flora HULTÉN (1944) expresses himself as follows: »*B. kenaica* is more closely related to the Kamchatkan white birch than to *B. resinifera* and may very well be regarded as a race of the latter». It is interesting to note that, according to a field botanist as familiar with the Alaskan as the Kamchatkan birch, the most westerly offshoot of the North-American birch forest is so like the most easterly Asiatic birch. However, the chromosome number of *B. papyrifera* v. *kenaica* is stated by WOODWORTH to be 70. A re-investigation of spontaneous material is here called for as well as in the case of *B. resinifera*, for which species no chromosome counts are available.

According to HULTÉN (1944), *B. papyrifera* v. *occidentalis* already occurs north of Juneau in Alaska. Further, in the west of North America there is another birch with an outstanding *verrucosa*-like character, *viz. B. fontinalis* SARG. = *B. occidentalis* (HOOK) FERNALD and perhaps more especially its form *B. f. Piperi* (SARG.) = *B. occidentalis* (HOOK) FERNALD var. *fecunda* FERNALD nom. nov. For *B. fontinalis* var. *Piperi* the chromosome number $2n = 28$ is given by WOODWORTH (1931). On geographical distribution grounds it seems most probable that *B. papyrifera* has been supplied with the *verrucosa* genome in the north-west of North-America. The East-American analogue of *B. fontinalis*, *viz. B. populifolia*, which is especially *verrucosa*-like, should not, either, be left out of the discussion. According to WOODWORTH (1931), this species is also diploid.

Continued experimental investigations will doubtless yield such valuable contributions to the systematization of the genus *Betula* that, together with taxonomical and geographical investigations, they should supply us with a firmly based conception of the structure and evolution of the genus. The author hopes to be able to contribute in this direction by analyses of several hybrids and by studies of a recently (JOHNSSON, 1948) gathered collection of rather representative material of North-American birches.

SUMMARY.

(1) The growth increment, morphological characters, meiotic conditions and fertility have been studied of the F_1 hybrids *B. verrucosa* ($2n = 28$) × *japonica* ($2n = 28$), *B. verrucosa* ($2n = 28$) × *papyrifera* ($2n = 84$), and *B. pubescens* ($2n = 56$) × *papyrifera* ($2n = 84$).

(2) All the three hybrids possess a manifest heterosis.

(3) In general features all the three hybrids are intermediate between the parental species.

(4) Meiosis is regular in *B. verrucosa* × *japonica* and *B. verrucosa* × *papyrifera*, while a high univalent frequency occurs in *B. pubescens* × *papyrifera*.

(5) The conclusion has been drawn that the genomes of *B. verrucosa* and *B. japonica* are structurally identical as well as that one of the *B. papyrifera* genomes is identical with the *B. verrucosa* one and that the other two genomes in 84-chromosomal *B. papyrifera* are identical with each other.

(6) Diplontic male-sterility occurs in *B. verrucosa* × *japonica* and in *B. verrucosa* × *papyrifera*. Both morphologically and functionally these two hybrids otherwise possess a good pollen. The pollen fertility of *B. pubescens* × *papyrifera* is considerably reduced.

(7) The female fertility is high in *B. verrucosa* × *japonica*, much reduced in *B. verrucosa* × *papyrifera*, and *B. pubescens* × *papyrifera* is practically female sterile.

(8) The importance of the fertility of the hybrids when use is made of F_1 populations with hybrid vigour in forestry practice is discussed.

(9) The chromosome number of *B. papyrifera* fluctuates between 70 and 84. The number $2n = 84$ is not restricted to *B. papyrifera* v. *occidentalis*.

(10) The taxonomic conditions within the genus *Betula* are discussed and the possibilities of experimental taxonomy yielding valuable contributions to the clarification of species-limits within a critical genus as this are suggested.

LITERATURE CITED.

1. Fernald, M. L. 1902. The relationship of some American and Old World birches. — American Journ. of Sc. IV. Series. Vol. XIV.
2. — 1945. Some North American Corylaceæ (Betulaceæ). — Rhodora 47.
3. Gunnarsson, J. G. 1925. Monografi över Skandinaviens Betulae. — Arlöv.
4. Helms, Anna og Jørgensen, C. A. 1927. Birkene paa Maglemose. — Bot. Tidsskr. 39.
5. Hultén, E. 1928. Flora of Kamtchatka and the adjacent islands. — Svenska Vetenskaps Ak. Handl. Serie 3, Bd II.
6. — 1944. Flora of Alaska and Yukon. — Lunds Univ. Årsskr. N. F. Avd. 2, Bd. 40.

7. JOHNSSON, H. 1941. Växtförädling av björk — mål och medel. (Breeding of birch — aim and methods.) — Medd. fr. För. f. växtförädl. av skogsträd (Svensk Papperstidning).
8. — 1944. Triploidy in *Betula alba* L. — Botaniska Notiser.
9. — 1945. Interspecific hybridization within the genus *Betula*. — Hereditas XXXI.
10. — 1948. Björk, asp och poppel från Nordamerika. (Birch, aspen and poplars from North America — an account of a journey.) — Medd. fr. För. f. växtförädl. av skogsträd nr 50 (Svensk Papperstidning).
11. KINDBERG, N. C. 1909. Om släktet *Betula*. — Botaniska Notiser.
12. LINDQUIST, B. 1947. On the variation in Scandinavian *Betula verrucosa* EHRH. with some notes on the *Betula* series *Verrucosa*, SUKACZ. — Svensk Bot. Tidskr. 41.
13. REHDER, A. 1948. Manual of cultivated trees and shrubs. — New York.
14. WETTSTEIN W. v. und PROPACH, H. 1939. Sichtungsarbeit zur Birkenzüchtung. — Der Züchter 11.
15. WOODWORTH, R. 1931. Polyploidy in the *Betulaceæ*. — Journ. Arnold Arb. XII.

CONTENTS.

Vegetative development 115
Morphological characters 117
Chromosome numbers 120
Course of meiosis 122
Fertility conditions 124
Discussion 129
Summary 133
Literature cited 134

SPINDLE ABNORMALITIES AND VARIATION IN CHROMOSOME NUMBER IN RIBES NIGRUM

BY *ANTERO VAARAMA*
STATE HORTICULTURAL INSTITUTE, PIIKKIÖ, FINLAND

I. INTRODUCTION.

THE decisive role of the spindle in the mechanisms which on the one hand regulate the congression of chromosomes at metaphase and on the other hand the anaphase separation of chromosomes has been explained during the last decades on the basis of different physical and physico-chemical hypotheses (CORNMAN, 1944; SCHRADER, 1944, 1947; ÖSTERGREN, 1945; ÖSTERGREN and PRAKKEN, 1946). Though it is impossible to decide in favour of any of these hypotheses, it remains an established fact that the nuclear cycle consists of a great number of separate, but chronologically strictly co-ordinated processes. The normal balance of these depends essentially on the normal course of the spindle cycle, *i. e.* on the exact adjustment of the timing and the regular structure and on the shape of the spindle.

Our knowledge of the factors which regulate spindle formation and of the factors which co-ordinate the activity of the spindle with the nuclear cycle is very imperfect. Artificial or spontaneous disturbances in the activity of these factors often offer a possibility of their closer study. In the literature we find in fact a number of studies which by means of such disturbances have shown the different nuclear phases to be to a great extent under genic control. Of the studies which throw light on the genic control of the spindle we may refer to the following: BEADLE (1931), WALD (1936), UPCOTT (1937), CLARK (1940), SMITH (1942), SWANSON and NELSON (1942), and SVÄRDSON (1945).

SVÄRDSON (*l. c.*) has named the genes regulating the nuclear cycle *mitotic genes* and supposes that they represent the »polygene» type. The term proposed is not exact, in so far as the activity of these genes is naturally not restricted to mitosis, but meiosis is equally under their control. Broadly speaking, cell division as a whole, including cell-plate formation, is controlled by this gene complex (*cf.* DARLINGTON and THOMAS, 1937).

The number of mitotic genes is evidently great (*cf.* BEADLE, 1932). Many of them have been observed to behave like typical oligogenes (*c. f., e. g.*, BEADLE, 1931), but it is possible that the assumption of the presence of polygenes (*in sensu* MATHER) is necessary for the understanding of the activity of these genes.

The interaction of mitotic genes is evidently so close that it is not possible to imagine disturbances, *e. g.* in the spindle, which would not simultaneously affect some other nuclear phase. It is, however, evident that the mitotic genes also comprise genes which regulate the timing, structure and activity of the spindle cycle.

Though various disturbances in nuclear division have been studied cytologically, too little attention has in my opinion been paid to spindle abnormalities. However, the literature contains certain cases which are impossible to explain except as results of spindle abnormalities. UPCOTT (1939 a) mentions *inter alia* certain super-reduced forms in the offspring derived from plants with certain spindle abnormalities. These forms, which are possibly more common than would be expected (*cf.* DARLINGTON, 1937, Table 27, p. 193), have a certain evolutionary significance. One might also mention, as an important factor, spindle abnormalities with simultaneous disturbances of other kinds in the division mechanism of the nucleus as inductors of sterility barriers between different forms. This fact has been especially stressed by SVÄRDSON (1945).

The material for the present study consists of several artificially raised *Ribes* strains (for their morphology and meiotic cytology, see VAARAMA, 1947) and their progeny. In their somatic cells spindle abnormalities are common. The observations on these disturbances and the resulting outlining of the problem are to a great extent similar to those presented by SVÄRDSON (*l. c.*). The present observations give further support to the conclusions drawn by DARLINGTON and THOMAS (1937) and SWANSON and NELSON (1942) in regard to the factors inducing compactness and polarity as well as genic control of the spindle.

II. MATERIAL AND METHODS.

The material comprises three different groups. (a) Five colchicine-induced tetraploid black currant bushes (*cf.* VAARAMA, 1947) in which mitoses have been studied in the meristem of young ovules; (b) c_2-progeny of a c_1-bush (strain B) comprising 139 individuals. In this case mitoses have been studied exclusively in the root-tip meristem.

The chromosome number in these bushes varies, $2n = 30—35$, the euploid number 32 being in the majority; (c) the progeny from a cross between two c_1-bushes (strain 9 × strain 10) consisting of 6 individuals. In this case, too, only root-tip mitoses have been studied. All these plants were grown in the Experimental Garden of the State Horticultural Institute (Piikkiö, Finland).

Fixations were carried out in the above-mentioned categories as follows: (a) short immersion in Carnoy fluid and thereafter fixation in craf-solution according to RANDOLPH; (b) the fixative used was craf as mentioned above; (c) partly craf-solution according to WEBBER, partly 2BD was employed.

For embedding paraffin was used and for staining crystal violet. From the root-tip material (c) a certain number of preparations were made using the Feulgen squash metod (DARLINGTON and LA COUR, 1947, p. 178). In this case the root-tips were fixed for 24 hours in acetic-alcohol.

III. RESULTS.

1. NORMAL CONDITIONS.

The somatic tetraploid chromosome complement of *R. nigrum* consists of small median and submedian chromosomes, which fixed in craf do not exhibit any morphological characteristics except satellites (Figs. 15—17). In regard to the satellites the tetraploid chromosome complement generally displays amphiplasty as all the plants of the c_2-generation, which were raised from seed of the B strain (most probably self-pollination) show only one satellite. In the c_2-individuals which originate from an artificial cross between two tetraploid strains, two satellites may also be found (Fig. 18).

When the fixations were made with 2BD and especially in autumn, when the temperature on several days had gone down to — 10° C and the soil temperature was near freezing point, the detailed morphological structure of the chromosomes became clearly visible. It could then be seen that the chromosomes possessed a great number of typical secondary constrictions (Figs. 1 and 18). In addition segments are found which are clearly narrower than the main chromosome body. These narrower segments are also visible after craf fixation in the same conditions (Fig. 14). It is possible that these segments represent the same type of heterochromatic differential segments as have been found after cold-treatment, *e. g.* in *Trillium* species (DARLINGTON and LA COUR,

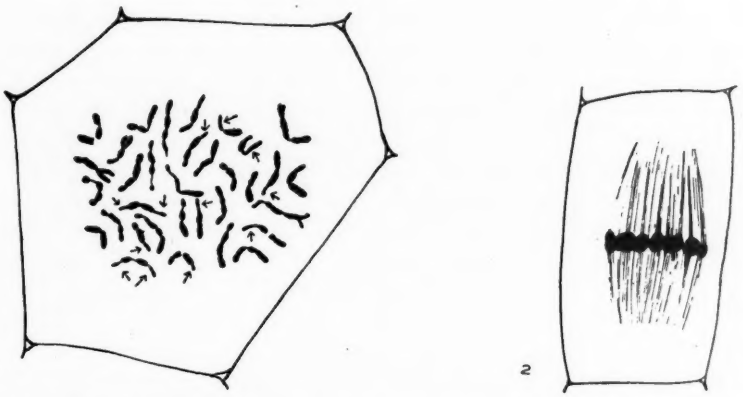


Fig. 1. A somatic metaphase plate from a root-tip ($2n = 33$) showing a great number of secondary constrictions. The narrower chromosome segments indicated with arrows. Fixed late in the autumn with 2BD. — × 3200. — Fig. 2. A metaphase side view from a root-tip cell in a diploid variety, showing the normal truncate type of the spindle. Craf fixation. — × 3200.

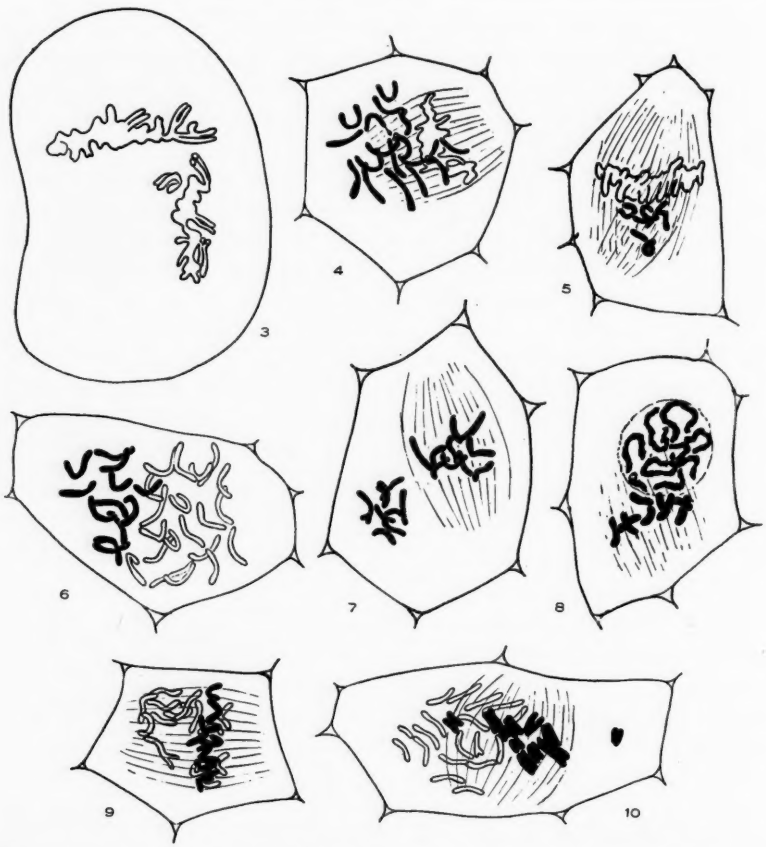
1940). Control experiments to prove this assumption definitely have not so far been made.

The form of the normal spindle is seen in Fig. 2, which is drawn from the root-tip of a diploid black currant variety. The spindle represents the truncate type in which the spindle fibres do not converge to form a sharp-pointed half-spindle cone. The polar region of the spindle thus remains diffuse.

2. SPINDLE ABNORMALITIES.

Since the material contained a great number of cells provided with variable chromosome numbers which were smaller than the normal tetraploid number, a mechanism ought to be found which would cause this reduction in the chromosome number. This phenomenon seems to depend on certain abnormalities observed in the spindle mechanism.

Most mitoses are quite normal irrespective of the chromosome number. Such divisions in which spindle disturbances are visible *in statu nascendi* are, however, relatively rare. Figs. 3—10 illustrate the type of mechanism for the reduction of chromosome number. It is based, in the plants in question, without exception *on the division of the spindle into two parts which act independently.* In consequence there is formed a double-plate metaphase, as the corresponding structure in the meiosis of *Primula kewensis* has been called by UPCOTT (1939 a).



Figs. 3—10. — Figs. 3—4. Split spindle. The spindle axes perpendicular. — Fig. 5. The axes of the sister spindles form an angle. — Fig. 6. The axes of the sister spindles parallel. — Fig. 7. The chromosome number of the plates is 18 in all, which shows that a split spindle has been formed anew in an already hypoploid cell. — Fig. 8. A great timing difference in the nuclear cycle of the two chromosome groups. The metaphase chromosomes super-contracted. — Fig. 9. One of the chromosome groups irregular. — Fig. 10. The anaphase movement in one chromosome group has been hindered. Explanation in the text. — Fig. 3 Feulgen squash, Figs. 4—10 craf fixation. — × 2500.

The axes of the sister spindles are either parallel (Fig. 6) or are situated at an angle to each other (Fig. 5). Quite often the axes form a right angle (Figs. 3, 4, 7 and 10).

In many cases it seems as if the metaphase plate were concave or

had undergone a torsion (Fig. 9). It is often difficult to say to which spindle those chromosomes belong which are situated along the line of the bend (Figs. 4 and 9). Separate chromosome groups (Figs. 3 and 7), may, however, be clearly observed even in the cases in which the spindle axes are parallel (Fig. 6).

It may be noted that real multipolar spindles, which are perhaps the most common type of spindle disturbances, and which are often encountered in connection with split spindles (*e. g.*, SASS, 1938; SWANSON and NELSON, 1942), have not been found in the present material.

The result of the action of two spindles in one cell seems to be the formation of four separate cells. The mode of cell wall formation has not been possible to follow in detail, but on the other hand no such structures as multinucleate or lobed cells, which arise owing to imperfect cell wall formation in connection with spindle abnormalities, have been observed (*cf.* DARLINGTON and THOMAS, 1937; SASS, 1938). Evidently cell wall formation is in principle similar to tetrad formation after the second meiotic division. These structures arise directly after mitosis owing to the activity of phragmoplasts (*cf.* DARLINGTON and THOMAS, *l. c.*).

3. TIMING ABNORMALITIES IN THE DEVELOPMENT OF SISTER SPINDLES.

The synchronization of spindle-formation and function with other processes of nuclear division is of paramount importance to the normal course of both mitosis and meiosis (*cf.* DARLINGTON, 1937, p. 408). Timing unbalance of the spindle is not generally directly visible. Its effects may only be inferred indirectly from the resulting disturbances in the other processes of nuclear division. It seems to be a common occurrence that the presence of split and multipolar spindles is connected with timing unbalance (*e. g.*, DARLINGTON and THOMAS, 1937; UPCOTT, 1937; SWANSON and NELSON, 1942).

As demonstrated by Figs. 3—7, the activity of the two sister spindles in the *Ribes* plants studied may be well synchronized. On the other hand they may also be at different stages (Figs. 8—10).

The timing difference of the sister spindles appears most often as a differential contraction of chromosomes in the different metaphase plates (Fig. 9). The contraction of the chromosomes in the one group must be regarded as relatively normal, whereas the chromosomes in the other group are clearly super-contracted. As SWANSON (1942) has shown experimentally in *Tradescantia*, the degree of spiralization of

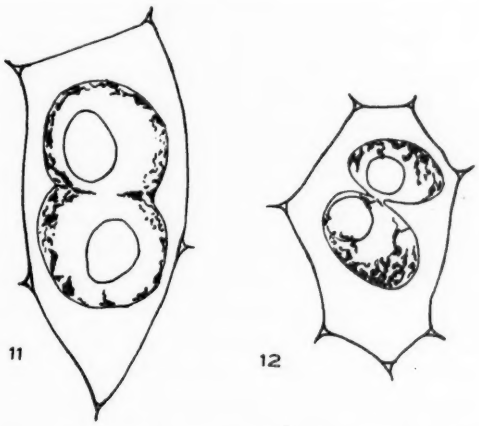
the meiotic metaphase chromosomes depends on the moment of formation of the spindle in relation to the spiralization phase of the chromosomes. When spindle-formation is delayed the meiotic metaphase chromosomes may be super-contracted. This is also the case in the male-sterile *Lathyrus* studied by UPCOTT (1937). In addition we must remember that a spindle as a rule consists of different elements: the external spindle which arises owing to the activity of centrosomes or other corresponding polarization factors, and the internal spindle which is formed in close connection with the centromeres (*c. f., e. g.,* SCHRADER, 1944). These are to a certain extent independent of each other and may show a timing unbalance. When the external spindle is delayed relative to the internal spindle, metaphase chromosomes will be super-contracted (DARLINGTON and THOMAS, 1937). It seems probable that timing disturbances analogous to the ones just described have also been effective in the *Ribes* mitoses, though it has not been possible to carry out a detailed analysis of these.

The difference in the chromosome groups as seen in Fig. 10 cannot in the author's opinion be explained exclusively on the basis of timing differences in the formation of spindles. The super-contracted chromosomes in the one group resemble fairly closely the c-pairs in mitotic metaphase after colchicine treatment (*cf., e. g.,* LEVAN, 1938). From this it may be inferred that the activity of the spindle is rather weakened.

This may be explicable on the same grounds as those on which EHRENBERG and ÖSTERGREN (1942) and EHRENBERG (1945) have explained the weakening of the spindle in the root-tip mitoses of *Salix* after acenaphthene- and cold-treatment. Their explanation is based on an assumption regarding the action of the spindle tactoid, *i. e.* on the length of its radius. The effective power of the tactoid strengthens when the radius increases, and *vice versa.* They have found in their experiments that the spindle curvature is increased owing to the treatment, which fact explains the simultaneous weakening of the chromosome movement. Now, the curvature of the spindle, as in the present case, may also increase owing to the space being too limited for the normal development of the spindle. Fig. 10 shows that the weakened spindle has been formed in a part of the cell which is too restricted for its normal length. The curvature of the spindle fibres has therefore been increased and the weakening of their action has prevented the anaphase movement of the daughter chromosomes. It is probable that this will result in all the chromosomes remaining in

the same nucleus just as in c-mitosis.

In Fig. 8 we see a case in which the timing difference between the two chromosome groups is considerable. The one group is in late prophase, while the other is in metaphase, the chromosomes in the latter being super-contracted. From this the conclusion must be drawn that the separateness of the chromosome groups in the two sister spindles may be derived, at least in certain cases, from earlier stages previous to the formation of the spindle, when the division of the spindle would seem most natural. This is supported by the fact that during the resting stage the nucleus may — though extremely rarely — already be constricted into two almost separate parts (Figs. 11—12). These nuclei resemble closely the constricted and lobed nuclei which have been described by SASS (1938) in the mitoses of barley treated with fungicides.



Figs. 11—12. Resting nuclei from root-tip cells. The shape of the nuclei indicates the existence of independent parts. Craf fixation. — × 3200.

It seems probable that these more or less separate parts of the nucleus are also autonomous in regard to their activity, in that their division cycles need not be exactly synchronous but may show a timing discrepancy of such a degree as that shown in Fig. 8.

4. VARIATION OF SOMATIC CHROMOSOME NUMBERS.

As previously mentioned, the somatic divisions of the plants studied show a great range of variation in their chromosome numbers. The lowest chromosome number found is but 4 (Fig. 13), *i. e.* $^1/_8$ of the most common normal chromosome number 32. All intermediate numbers between the normal number and four have been observed in the present material.

The cells which possess deviating chromosome numbers have been found to be dispersed among the tetraploid cells. Those with the lower

numbers have not been found to be concentrated on any of the different meristematic zones of the root-tip, *i. e.* periblem, plerome or dermatogen. Neither has it been observed that any large cell groups consist of cells provided with a certain chromosome number. The decrease of the chromosome number has thus not given rise to any chimaeras of the tissues. As, *e. g.*, Figs. 7 and 8 illustrate, a split spindle may also arise *de novo* in cells in which the chromosome number has already decreased. This naturally prevents the occurrence of larger cell groups with the same chromosome number.

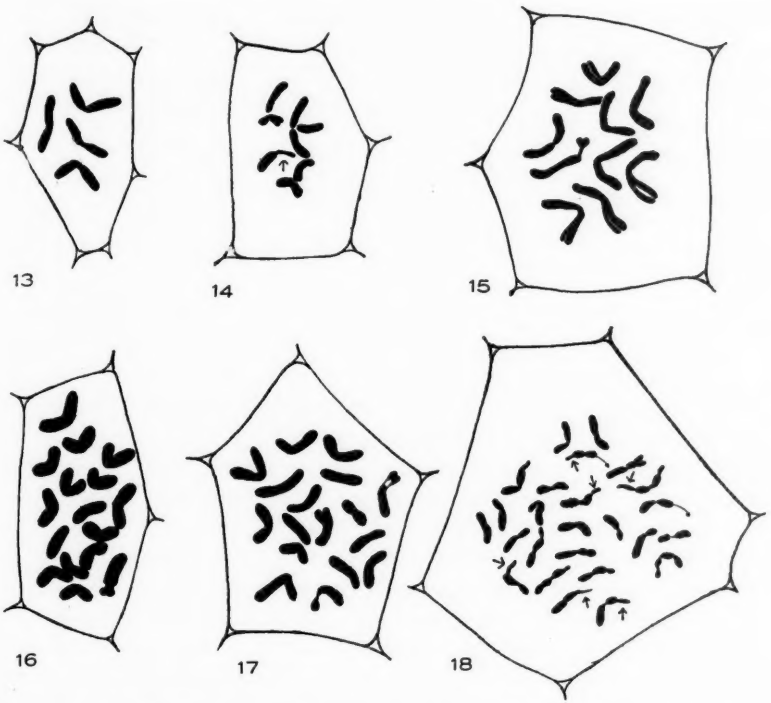
As the mitotic chromosome numbers counted in the young ovules belong to the c_1-generation of colchicine-treated plants, they might be explained as representing mixoploidy, so commonly met with in such plants, which results from the uneven action of colchicine. This seems, however, to be improbable, since the cells with the lower chromosome numbers are here dispersed among normal cells, and no chimaerical cell groups are found. Chromosome reduction in the ovules studied has taken place in the meristem through a split spindle.

The frequency of the lower chromosome numbers as compared with the normal ones is difficult to estimate. Evidently their number varies in different bushes. According to approximate estimations, the number of cells with lower chromosome numbers is about 3—10 % from the total number of cells suitable for chromosome counts. For the sake of comparison we may mention that UPCOTT (1939 a) has found 8 % super-reduced PMC's in *Primula kewensis*, in which the mechanism is fairly similar.

As already mentioned, the course of meiosis is quite regular in all cases in which a split spindle has not been formed, independent of the chromosome number. In Figs. 13—18 we see metaphase plates with different chromosome numbers, which illustrate this. In the contraction of the chromosomes certain differences may be observed (*e. g.*, supercontracted chromosomes in Fig. 16) which show that the timing balance of the spindle is not always quite normal.

A pertinent phenomenon visible in all root-tip meristems studied is the occurrence of numerous dead and degenerated cells amongst the living cells. This suggests that a certain part of the cells, probably among those that have obtained the decreased chromosome number, have not been viable.

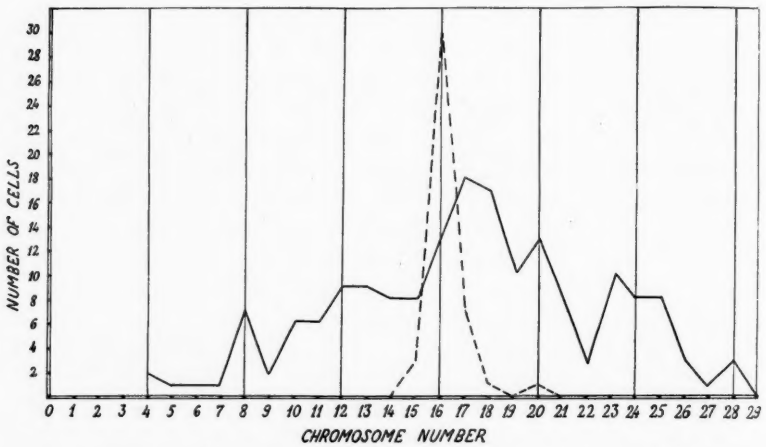
The relatively large amount of material available afforded an opportunity of studying the frequency of cells with different decreased chromosome numbers. As material only such metaphase plates, or in



Figs. 13—18. Different hypoploid metaphase plates from root-tip cells. 4, 8, 10, 16, 17 and 25 chromosomes. In the cells which have suffered from cold the narrower segments are indicated with arrows. Figs. 13—17 craf, Fig. 18 2BD fixation. — × 3200.

certain cases late prophases, have been used as were without doubt uninjured and in which the chromosome number could be determined with certainty. The most abundant material was provided by the root-tip mitoses [bushes of the c_2-generation, see Chapter II, (b) and (c)] from which 182 plates with deviating chromosome numbers have been counted. Also in the young ovules cells with hypoploid chromosome numbers these could be determined in 42 cases [bushes of the c_1-generation, see Chapter II, (a)].

The frequencies of the different chromosome numbers, separately for root-tip and ovule mitoses, are presented in Graph 1. The graph representing the frequency of the decreased chromosome numbers in root-tip mitoses is binomial in its main features. This implies that most commonly, when two sister spindles are formed, each spindle receives



Graph 1. Graph representing the frequency of different abnormal chromosome numbers counted (a) in cells of root-tip meristem (continuous line) and (b) in cells of young ovules (broken line).

the same number of chromosomes. Chromosome groups with widely differing numbers are more rare. The dispersion may be regarded as very great. The graph of the ovule mitoses shows quite clearly, though the material in this case is much smaller, that the dispersion of the different chromosome numbers is much smaller. The division of the tetraploid number into two similar groups is by far the most common. The other numbers found indicate only slight deviations from this average.

The statistics of the chromosome numbers in the root-tip mitoses exhibit, however, certain significant deviations from the binomial distribution. From them, we see that in the root-tip graph *a peak occurs at every number divisible by four*, which shows these numbers to be more frequent than could be expected from a random distribution. We may suppose that the greater frequency of these chromosome numbers depends on the better viability of cells with chromosome numbers divisible by four. Since a great number of cells with less balanced chromosome numbers die, which is shown by the occurrence of degenerated cells in the meristem, the share of the viable cells becomes greater than normal.

It seems probable that amongst these most vital chromosome combinations those in which the chromosome number is 16 or close to

this normal number in *R. nigrum* are in addition to being the most common ones also the most viable. This is supported by the fact that dead cells have not been observed in the young ovules in which the chromosome number varies around 16.

IV. DISCUSSION.

1. EVIDENCE FROM THE FREQUENCY OF DIFFERENT CHROMOSOME NUMBERS.

When considering the varying somatic chromosome numbers found in the *R. nigrum* studied, the question arises as to how it can be possible that even such chromosome groups as contain only four chromosomes, the halved normal haploid number 8, may have an undisturbed nuclear cycle. This fact is rather surprising in the light of the opinion based on extensive observations that the haploid number of chromosomes is the smallest possible unit allowing a normal development of a cell. The definition given by DARLINGTON and THOMAS (1941, p. 127) is based upon this opinion: »The ordinary chromosomes making up the haploid set of an individual or a species are all necessary for the regular development of every tissue and of every isolated cell».

SVÄRDSON (1945, p. 96), who in his excellent study on the cytology of the Salmonoids has been faced with a corresponding problem, has presented four possible solutions: (a) »The mitosis is not subjected chiefly to genetic control, but is mainly directed by the cytoplasm.» (b) »Nuclei with normal chromosome sets are capable of controlling the mitoses of the other adjacent nuclei which lack a normal chromosome set.» (c) »The mitotic genes are concentrated to a few chromosomes, and each chromosome may, possibly, have a set of genes permitting the mitotic division.» (d) The fourth possibility implies that the species regarded as diploid actually is polyploid, *i. e.*, the set of chromosomes is smaller than expected. Doubtless all the possibilities mentioned must also be considered in the present case.

The first assumption is very improbable, which is shown by the fact that disturbances in the nuclear cycle are inherited through the male gametes (SWANSON and NELSON, 1942; SVÄRDSON, 1945). The occurrence of spindle disturbances in the c_2-generation of *R. nigrum* makes it very probable that they are inherited through the chromosomes, though it has not as yet been possible to verify this assumption by means of crossing with a normal individual.

The greater frequency and viability of certain chromosome combinations in *R. nigrum*, and especially the degeneration of a number of cells, proves in my opinion that viability depends on the quality of the chromosomes and not on the surrounding plasm.

The second hypothesis is not exclusively theoretical, since in the literature a number of cases have been described in which normal cells apparently support cells with lower chromosome numbers which have arisen owing to spindle disturbances. BARBER (1941) has found such cases in the pollen grains of *Uvularia* as well as in the complex pollen grains of certain orchid species (1942). KOLLER (1947) has stated that malignant tumour cells with lower chromosome numbers are able to exist with the aid of the intensive physiological activity of the neighbouring cells. In regard to the pollen grain cases mentioned above it has been found that the isolation provided by the cell walls has remained very weak, allowing a free interaction between the cells. A situation thus obtains which is normal in animal tissues. An indication of the interaction of the cells is furnished by the exact synchronization of the nuclear cycle.

In the fish species studied by SVÄRDSON (1945), in which as in *R. nigrum* synchronization is lacking, certain cases occur in which the whole embryo consists of cells with lower chromosome numbers. In these cases the normal cells cannot have any compensating activity. The cell walls of *Ribes* have a normal structure, preventing any free interaction. This is shown by the fact that the cells are autonomous in their division cycle. In addition to these data the occurrence of dead cells also speaks against this assumption, since these cells would certainly get support from the adjacent cells, if this should be possible.

The assumption that a cell provided with less than the haploid number of chromosomes would despite this contain all the necessary mitotic genes seems improbable. We have reason to suppose that the number of mitotic oligogenes is considerable. This is also indicated by the observations made on the more extensively analysed *Zea* (BEADLE, 1932; CLARK, 1940). This is hardly remarkable, if we remember how many different processes make up the nuclear cycle.

The interaction of the mitotic genes is very complicated and it is possible, although exact information is lacking, that their normal balance is controlled by modifiers having the nature of polygenes. In addition we know that the normal course of the nuclear cycle depends on the exact adjustment of the nucleic acid metabolism, which in its turn is determined by the heterochromatic chromosome parts (DAR-

LINGTON, 1947; KOLLER, 1947). These facts indicate that the normal balance of the nuclear cycle is determined by the whole chromosome set.

On the other hand, many studies show that especially in meiosis small individual chromosome groups, even separate bivalents, may form a spindle, divide and form micronuclei which are separate from the main part of the chromosomes (*cf.* FRANKEL, 1937). In these cases we must remember that the processes in question take place within a single cell, in one physiological unit inside the cell wall, where the independence of these processes is rather questionable, as the compensation between the different parts is unhindered. This is supported, *e. g.*, by the observations of CLARK (1940) that the multinucleate gametes in maize that arise owing to a divergent spindle in meiosis are viable if the micronuclei together contain one complete chromosome set. As a rule, the micronuclei formed in the meiosis of plants die when the cell wall has developed around them. Such polymitotic divisions described by BEADLE (1931) in which the chromosome number may be reduced to one or two chromosomes also take place in the space inside the pollen grain wall. Divisions of this kind cannot be regarded as independent. The cases in which the hypoploid cells have been observed to function by means of the compensation of the neighbouring cells have been described above.

We must not forget in this connection that in different organisms the physiological differentiation of the chromosomes is very different. In extreme cases, as *e. g.* in *Hyacinthus*, every chromosome contains a well-balanced genic complex (DARLINGTON and MATHER, 1944), which makes individuals with widely differing chromosome numbers viable. A reverse example is furnished, for instance, by the tetraploid spontaneous apple variety Hibernal, in which even rather large chromosome groups eliminated at meiosis are not able to form micronuclei (VAARAMA, 1948). However, even in those cases in which the physiological differentiation of the chromosomes is weak subhaploid cells have not so far been observed to be viable.

Finally we must take under consideration to what degree the last assumption holds true, *i. e.* that the viability of hypoploid cells in *R. nigrum* could depend on cryptic polyploidy, in other words on the existence of a smaller basic chromosome number than has been supposed. The statistics on the frequency of different chromosome numbers seem in my opinion to speak strongly in favour of this assumption. Since the smallest chromosome number found is 4, we may

regard it as probable that this number represents the original haploid chromosome number. Another observation supporting this view is that chromosome numbers divisible by four, or numbers close to them, are more frequent than could be expected on random distribution and thus such groups are evidently more viable. It is probable that when the split gives rise to different chromosome numbers, the chromosomes divide between the two groups rather much at random. There is a high probability that the small chromosome groups arising do not contain the complete set of 4 chromosomes. Such cells are not viable, but degenerate. The dead cells found in the root-tip meristem result from such chromosome combinations. Larger chromosome groups are much more likely to contain full chromosome sets and are thus viable to a much higher degree. This is shown by the fact that young ovules in which small chromosome groups do not occur do not contain dead cells either. Naturally, amongst the larger chromosome combinations, too, those which have a chromosome number that is an exact multiple of the chromosome set or fairly close to such a multiple are best balanced and most viable.

On the basis of the data presented above it may be regarded as probable that *the original basic chromosome number of Ribes is 4* and that the present species, in which without exception the number $2n = 16$ has been observed, are derived tetraploids. This assumption is in accordance with the hypothesis of STEBBINS (1938, 1947) that many woody plants which have been regarded as diploid are actually polyploid.

Speciation in the original forms with $2n = 8$ chromosomes must have occurred very long ago. The original forms have apparently completely disappeared and the whole genus, which is often regarded as an independent family, is monotypic in regard to its life-form, consisting of woody shrubs. Herbaceous types are completely absent. In the speciation process hybridization combined with allopolyploidy seems to have been the most probable agent. This is indicated by the observations made on the meiotic chromosome pairing in certain recent sterile *Ribes* hybrids, *e. g. R. Culverwellii* (MEURMAN, 1928), in which the most frequent number of bivalents is 4 (on the average 4,55). From this may be inferred the existence of one common set of four chromosomes in the complements of *nigrum* and *grossularia*, while the other chromosomes seem to be less related. The question of what has caused the polyploidy in the genus *Ribes* to have remained at the tetraploid level cannot be solved for the present. Reference may in this

connection be made to the assumption of GUSTAFSSON (1948) that higher polyploidy is to a great extent connected with asexual modes of reproduction, which in the genus *Ribes* are rather poorly developed.

2. CAUSES AND RESULTS OF THE SPLIT SPINDLE FORMATION.

Earlier it was assumed that the nuclear division mechanisms of organisms might be sharply divided into two separate groups depending on whether a polarity factor called the centrosome was present (animals, lower plants) or absent (higher plants). At present this view is more or less abandoned, as the observations show that a mechanism regulating the polarity is also present in those cases in which it was previously supposed to be lacking (DARLINGTON and THOMAS, 1937). The difference lies in the fact that either it is formed by a corpuscular, stainable body, the centrosome, or it is diffuse. This opinion has already been presented by BELAR (1928).

Normal spindle formation is the result of the interaction of an external factor, the polarity factor, and the internal factor, the centromere. The mutual adjustment of these is, as mentioned earlier, important. This adjustment is of adaptive nature, as shown by many special cases described, for instance, in SCHRADER's review (1944).

External factors, the centrosome as well as the diffuse polar region exert their activity outside the nucleus, being able to form a spindle, and even cell wall, without a nucleus or a simultaneous nuclear division connected with the spindle (spindles in egg fragments devoid of a nucleus, FRANKHAUSER, 1934; formation of cell wall in nuclei-free cells, KAGAWA and CHIZAKI, 1934; CHIZAKI, 1934; unattached spindles, DARLINGTON and THOMAS, 1937). The polarity factor is, however, not of plasmatic origin, but is formed and inherited through the nuclear cycle. The function of the polarity factor seems accordingly to be strictly genically controlled. CLARK (1940) has found that a certain recessive gene in maize induces the development of a divergent spindle instead of the normal convergent one. The divergency of the spindle depends evidently on the abnormal diffuseness of the polar region. The gene in question consequently affects the activity of the polar region. The studies of WALD (1936) on *Drosophila simulans* show that a mutated claret gene gives rise to certain spindle disturbances, and that the absence of the second or third chromosome completely prevents the formation of the spindle. The genes influencing spindle development thus lie in the chromosomes mentioned. In this connection we may also consider the observations that prove the chromosomal inheritance

of split and incompact spindles, which may be regarded as special disturbances of the polar region (DARLINGTON and THOMAS, 1937; PARASARATHY, 1938; SWANSON and NELSON, 1942). As mentioned in the previous chapter, this also seems to be the most probable explanation in regard to *R. nigrum*.

The formation of the split spindle always implies that the polar region divides into separate parts. Now, we have two possibilities. This division takes place either simultaneously with the appearance of the spindle or the first impulse towards the formation of separate spindles may have already occurred at an earlier stage. The observations on *R. nigrum* show that at least in certain cases the latter alternative must hold true. The shape of the resting nuclei indicates a division into separate parts, which leads in the extreme cases to an »amitotic» constriction of the nucleus into almost separate parts.

On the basis of the above data we may draw the conclusion that even in the normal nucleus the parts must possess a latent autonomy. This is, however, covered by a controlling factor which in the normal nucleus regulates the co-operation of the parts. The nature of this factor is so far obscure, however. When its activity is in some way disturbed, the existence of the independent parts is revealed.

The most natural assumption would be that the occurrence of these parts is based on the haploid chromosome sets. As was established in the previous chapter, the haploid chromosome set must be regarded as the smallest possible unit which renders an individual cell, tissue or organism viable. This character of a' uniform complex is evidently maintained in the haploid set in connection with other chromosome sets as well. Polyploidy and numerous nuclear fusions formed through disturbances in cytokinesis (*cf.*, *e. g.*, SMITH, 1942) show, however, that even a great number of chromosome sets may co-operate regularly. In these cases we must assume that the different chromosome sets are similar to a certain degree.

If, however, a nucleus receives chromosome sets which already as such are unbalanced, or if the different sets are so dissimilar that they cannot be adapted to the main control of cell division, the cell mechanism is very liable to different irregularities (*cf.* GOODSPEED, 1946). The lack of general control may give rise to the independent behaviour of the set in the resting nucleus, which in turn results in the formation of independent sister spindles in the same cell. Thus, the somatic complement of the »tetraploid» *R. nigrum* comprises 8 separate chromosome sets, the original basic number being disclosed by the disturb-

ances. This should furnish a possibility for several sister spindles to occur at the same time. For some reason only two sister spindles are formed, in some cases successively.

If we consider the reasons for the disturbance of the normal balance of the chromosome set, the following factors must be taken into account. Firstly, mutations may occur in the complex of mitotic genes. If they are recessive their activity is revealed in the heterozygous state, as described, *e. g.*, by BEADLE (1931, 1932), WALD (1936), CLARK (1940), and SMITH (1942). Secondly, structural changes in the chromosomes must be taken into consideration. The results may in this case be different. First we have to consider the position effect of the genes. Secondly, if we assume that the balance of the mitotic genes depends on modifiers of polygene character, their number and mutual relations may be changed. Thirdly, the balance of nucleic acid metabolism is an important factor influencing the cell cycle. As this in its turn depends on the amount of heterochromatin, structural changes in the chromosomes may thus give rise to variation in the nucleic acid content of the cell. Since polygenes are supposed to lie in the »inert» heterochromatic parts (MATHER, 1945), disturbances in polygenic control and variation in the nucleic acid charge are closely connected. Whether polygenes and the activity of heterochromatin in the protein metabolism remain in causal relation is so far, however, an open question.

The observations of DARLINGTON and THOMAS (1941) on abnormal polymitoses in the pollen grains of *Sorghum* have shown that they are caused by the heterochromatic B-chromosomes. In many cases a similar positive correlation has been established in regard to the B-chromosomes of *Anthoxanthum* (LIMA DE FARIA, 1947). Most clearly the relation between the amount of heterochromatin and the excess of nucleic acid and different disturbances in cell divisions is to be seen in the chromosome conditions of malignant tumours. As early as in 1931 LEVINE described a number of disturbances characteristic of tumour cells. Such disturbances have been analysed more closely by CASPERSSON and SANTESSON (1942) and KOLLER (1943), who have explained them as depending on the nucleic acid content.

A supernormal nucleic acid charge gives rise to a more intense cell activity. The synchronization of the different processes of which cell-division consists is disturbed, which has a great number of different results. Most of the division disturbances known may be explained on the basis of an abnormal timing balance, although so far a clear

connection with variation in the nucleic acid charge has only been found in few cases.

If we consider the spindle disturbances in the *R. nigrum* bushes now in question, it seems evident that the balance of the mitotic genes is unsettled. There now arises the question what the cause of these irregularities can be, and in which of the categories mentioned above they may be included. The observations available do not warrant any final conclusions being drawn. We may, however, present a working hypothesis on the basis of the present observations.

If we have a gene mutation, it may have occurred in the complex of the mitotic genes either spontaneously or by means of such agents as have been found to cause mutations. Since the nuclei acid charge of the cell is genically controlled (KOLLER, 1943), changes in the latter may also result from mutations. Structural re-arrangements in the chromosomes, too, may be either spontaneous or induced.

The occurrence of spontaneous gene mutations is improbable, as spindle disturbances occur in all the c_1-shrubs studied. Of the inducing agents the most probable seems to be colchicine, by means of which all the c_1-bushes have been brought about.

The activity of colchicine and of other similarly active agencies has been regarded as influencing above all the spindle system together with the polarity factor (*cf.* LEVAN, 1938; BARBER and CALLAN, 1942). The activity of the former is weakened, which process may be compared with narcosis, being reversible (*cf.* ÖSTERGREN, 1944). The genotype is accordingly not permanently changed.

Certain cases show, however, that in connection with c-mitosis different kinds of structural alterations in the chromosomes may occur. In *R. nigrum*, in which this phenomenon is found, I have assumed this process to take place in connection with a certain type of c-mitosis, called by BARBER and CALLAN (1942) the ball-metaphase (VAARAMA, 1947).

The occurrence of permanent spindle disturbances by means of colchicine may naturally be explained in two ways. Colchicine gives rise either to gene mutations in the complex of mitotic genes or to structural changes in the chromosomes. The fact that only very few compounds have been found to induce mutations itself speaks against the mutative character of the irregularities. As the most important of such substances may be mentioned mustard gas (KOLLER, 1947; DARLINGTON and KOLLER, 1947). Although the discovery of possible mutations in *R. nigrum* is difficult owing to the high degree of hetero-

zygosity, the present material has not exhibited mutations, at any rate of the type arising spontaneously or experimentally in plants.

A better-founded explanation seems to be that structural changes in chromosomes have resulted in the unbalance of the mitotic genes. DARLINGTON and LA COUR (1940) have shown that the heterochromatic chromosome segments are especially liable to breakage during metaphase, when undercharged with thymonucleic acid, and consequently undergo structural re-arrangements. Since it seems probable that heterochromatic segments form an important part of the somatic chromosomes in *R. nigrum*, it is understandable that chromosomes break during c-mitosis, the break occurring most often in the heterochromatic segments. C-mitosis thus gives rise to a great number of cells which deviate from the normal ones in regard to the amount of heterochromatin. Accordingly, nucleic acid synthesis varies quantitatively, and this in turn is revealed by the disturbances in the division of the cell. Especially those irregularities which have been explained as due to insufficient timing adjustment seem to be explicable on this basis.

The relation to nucleic acid metabolism is, however, obscure as regards certain phenomena. The occurrence of autonomous chromosome groups may especially be mentioned. In regard to such phenomena we can only refer to changes in the gene balance resulting from structural re-arrangements in chromosomes. Since structural changes occur most frequently in the heterochromites, the result is a break-down of the modifier system which regulates the co-ordination of the genes. It might be thought that each of the haploid chromosome sets must be balanced to be able to co-operate in their synchronization. This naturally does not exclude the possibility that the main control can be re-established as a result of adaptive changes and selection.

From the fact that spindle disturbances do not occur in all cells the conclusion may be drawn that insufficient genic balance does not necessarily involve disturbances. When disturbances occur a number of different factors, including the external factors, must be effective in regard to the physiological balance of the cell. Since buffering against these factors is weakened, the opportunities for disturbances to occur have increased in the cases in which conditions are favourable for them. SWANSON and NELSON (1942) have explained corresponding observations on *Mentha* by assuming that the gene giving rise to spindle disturbances has an incomplete dominance.

To complete the picture given above we may consider how far the

hypotheses recapitulated are compatible with disturbances in the mechanism of nuclear cycle described in the literature.

In hybridization two more or less different chromosome sets come together. The mitotic genes of these two sets are often not balanced with one another, which easily leads to different abnormalities in nuclear division. For instance, those irregularities which have been observed by DARLINGTON and THOMAS (1937) in a trisomic derivative from a cross between *Festuca* and *Lolium* may be explained on this basis. The same holds true of the *Salmo* hybrids studied by SVÄRDSON (1945). This explanation has, indeed, been presented in both these papers. In *Primula kewensis* (UPCOTT, 1939 a) a regular chromosome pairing has been established through allopolyploidy, which, however, has failed to bring about the balance of mitotic genes. The establishment of the normal balance is in this case prevented by occasional multivalent formation and the resulting uneven segregation of chromosomes.

An interesting example is further furnished by the spindle disturbances in the hybrids between the A- and B-races of *Drosophila pseudoobscura*, which have been studied by DOBZHANSKY (1934). The chromosome complements of these races differ particularly in regard to the heterochromatic and inert *Y* chromosomes. Considering that MATHER (1945) assumes the *Y* chromosome to be an important site of polygenes in *Drosophila*, we might think that in this case hybridization gives rise to unbalance in the nucleic acid metabolism and in the modifier system, which in turn results in disturbances of the nuclear mechanism.

The hybrid *Mentha piperita* × *spicata* described by SWANSON and NELSON (1942) represents a special case. *M. piperita* is a hybrid which, however, is balanced in regard to mitotic genes. *M. spicata*, again, is a pure species showing spindle disturbances, which are inherited by the hybrid. In this connection we must note a considerable difference between these two species. The figures of SWANSON and NELSON (*l. c.*) show that *M. spicata*, unlike *M. piperita*, has heterochromatic segments. Spontaneous structural changes may accordingly easily take place in *M. spicata*, which seems to be a rather complicated polyploid in origin.

The spindle abnormalities in *Oryza* described by PARASARATHY (1938) may most probably de derived from structural changes or mutations which have been brought about by x-ray treatment of the parental generation. The mitotic disturbances in the cold-treated gwyniad embryos studied by SVÄRDSON (1945) show that a treatment of this kind may induce permanent changes in the balance of mitotic genes.

Disturbances in the mitotic gene activity may best be seen in pollen grain mitoses, in which only the primarily or secondarily haploid chromosome set is present. The studies of DARLINGTON and THOMAS (1941) and LIMA DE FARIA (1947) on the activity of the B-chromosomes in *Sorghum* and *Anthoxanthum* have already been mentioned. In these papers reference is also made to other cases in which supernumerary mitoses in pollen grains occur in connection with artificial or spontaneous genic unbalance (DE MOL, 1923; SAX, 1935, 1937; UPCOTT, 1939 b; DARLINGTON and JANAKI AMMAL, 1945).

Finally the question arises, what significance the occurrence of hypoploid cells may have for the formation of gametes and the subsequent generation. When the microsporogenesis of the »tetraploid» *R. nigrum* was being studied (VAARAMA, 1947), no attention was paid to the possible occurrence of hypoploid gametes in the sectioned material. In the progeny of the »tetraploid» B-strain, however, amongst 139 c_2-shrubs studied 3 »diploids» with $2n = 16$ were encountered. Their occurrence is without doubt connected with the lowered chromosome numbers which have been found in the mitoses of the young ovules. C_2-shrubs have as yet not flowered and consequently no observations have been made concerning them.

On the strength of the split spindles in *Primula kewensis* UPCOTT (1939 a) has assumed that in the progeny of a selfed plant certain individuals having deviating chromosome numbers (NEWTON and PELLEW, 1929) might be derived from a super-reduction of the chromosome number as a result of a division of the spindle. She also refers to the possibility that sporadic unexpected gametes, tissues and individuals provided with lower chromosome numbers could have arisen in the same manner. In this connection we may mention the »haploid» PMC's found by LOVE (1936) in *Triticum vulgare*.

It seems an important question, to what degree the autonomy of the chromosome sets in the resting nuclei leads to extraction of the chromosome belonging to a certain haploid set into a separate group. In other words, how far the chromosome groups in the different sister spindles have a non-random make-up of more or less complete haploid sets. Such a possibility is suggested by certain observations on the haploid tissues in *Drosophila melanogaster* (BRIDGES, 1925; MC KNIGHT, 1937), which may have arisen through a split spindle mechanism (*cf.* UPCOTT, 1939 a). These tissues exhibit certain recessive characters which are inherited exclusively from the male. This can be taken as an indication of the elimination of the maternal chromosome set. Such

autonomy of the different haploid sets has been found to be normal in the fly *Sciara* (METZ, 1933), being in this case, however, connected with an aberrant nuclear mechanism.

Though it may be possible that the autonomy of the different chromosome sets leads to a tendency of the sets to separate, it seems improbable that this actually could be realized. For it is difficult to imagine a mechanism which could separate the chromosome at this stage. In addition it is evident that when the metaphase plate is divided between the different spindles the chromosomes are distributed quite at random. It seems more probable that the randomly formed chromosome groups are subject to a strong selection with the consequence only the suitable gene combinations are able to form separate tissues, viable gametes and thus viable super-reduced individuals. In this I agree completely with the view of UPCOTT (1939 a).

In any case the above observations on *R. nigrum* make it evident that the opinion of the complete reversibility of c-mitosis, resulting from colchicine and other polyploidy-inducing treatments, must be revised. Attention ought to be paid to the permanent effects which these agents may have on the treated chromosome complements. The result may be the formation of individuals provided with new deviating gene combinations which may have a certain evolutionary significance.

I am greatly indebted to the Director of the Horticultural Institute, Professor Dr. O. MEURMAN, for good advice and criticism during the work, and to Dr. EEVA THERMAN-SUOMALAINEN for the elaborate help when preparing the manuscript.

SUMMARY.

(1) A number of colchicine-induced tetraploid *Ribes nigrum* bushes ($2n = 32$) and their c_2-progeny raised from seed have been studied. The chromosome number in the somatic cells has been found to be very varying (4—32).

(2) The formation of two separate and independent spindles in one cell has been established as the cause of the diminution of the tetraploid number.

(3) The autonomy of the separately dividing chromosome groups has been found as early as in the resting nucleus, which in this case is more or less constricted.

(4) In the mitotic cycles of the two separate chromosome groups disturbances in synchronization have often been found.

(5) The statistics of the frequency of the different somatic chromosome numbers show that in its main features the distribution is binomial, the highest frequency lying at the diploid number. It may, however, be observed that all numbers divisible by four are more frequent than might be expected on a random distribution.

(6) The conclusion that the original basic number of the genus *Ribes* is 4 instead of 8 and that the recent species are derived tetraploids has been drawn from the viability of the cells with 4 chromosomes and the frequency of different numbers.

(7) The formation of split spindle and the timing disturbances in the divisions are assumed to be connected with structural differences in the chromosomes, arising in c-mitosis. Chromosomes are then broken especially in the heterochromatic segments. The amount of heterochromatin is thus variable and consequently the nucleic acid metabolism is unbalanced. It seems also to be of importance that structural changes disturb the balance of the mitotic genes which regulate the nuclear cycle.

(8) The diploid bushes ($2n = 16$) present in the progeny of the tetraploid bushes ($2n = 32$) show that the hypoploid cells also affect the offspring. A strong selection seems, however, to be effective in this respect.

LITERATURE CITED.

1. BARBER, H. N. 1941. Chromosome behaviour in *Uvularia*. — Journ. of Genetics 42: 223—257.
2. — 1942. The pollen grain division in the Orchidaceae. — Journ. of Genetics 43: 97—103.
3. BARBER, H. N. and CALLAN, G. H. 1942. The effect of cold and colchicine on mitosis in the newt. — Proc. Roy. Soc. London B 131: 258—270.
4. BEADLE, G. W. 1931. A gene in maize for supernumerary cell divisions following meiosis. — Cornell Univ. Agr. Exp. Stat. Mem. 135: 1—12.
5. — 1932. Genes in maize for pollen sterility. — Genetics 17: 393—412.
6. BELAR, K. 1928. Die cytologischen Grundlagen der Vererbung. — Handb. Vererbungswiss. I. Berlin.
7. BRIDGES, C. B. 1925. Haploidy in *Drosophila melanogaster*. — Proc. nat. Acad. Sci. Wash. 11: 706—710.
8. CASPERSSON, T. and SANTESSON, L. 1942. Protein metabolism in the cells of epithelial tumours. — Acta Radiologica. Suppl. 46: 1—105.
9. CHIZAKI, Y. 1934. Another new haploid plant in *Triticum monococcum* L. — Bot. Mag. Tokyo 48: 623—628.

10. CLARK, F. J. 1940. Cytogenetic studies of divergent meiotic spindle formation in *Zea mays*. — Amer. J. Bot. 27: 547—559.
11. CORNMAN, I. 1944. A summary of evidence in favor of the traction fiber in mitosis. — Amer. Nat. 78: 410—422.
12. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
13. — 1947. Nucleic acid and the chromosomes. — Symp. Soc. Exp. Biol. 1. Nucleic acid: 252—269.
14. DARLINGTON, C. D. and JANAKI-AMMAL, E. K. 1945. Adaptive isochromosomes in *Nicandra*. — Ann. Bot. N. S. 9: 267—281.
15. DARLINGTON, C. D. and KOLLER, P. C. 1947. The chemical breakage of chromosomes. — Heredity 1: 187—221.
16. DARLINGTON, C. D. and LA COUR, L. 1940. Nucleic acid starvation in chromosomes of *Trillium*. — Journ. of Genetics 40: 185—213.
17. — and — 1947. The handling of chromosomes. 2nd ed. — London.
18. DARLINGTON, C. D. and MATHER, K. 1944. Chromosome balance and interaction in *Hyacinthus*. — Journ. of Genetics 46: 52—61.
19. DARLINGTON, C. D. and THOMAS, P. T. 1937. The breakdown of cell division in a *Festuca—Lolium* derivative. — Ann. Bot. N. S. 1: 747—761.
20. — and — 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. — Proc. Roy. Soc. London B 130: 127—150.
21. DE MOL, W. E. 1923. Duplication of generative nuclei by means of physiological stimuli and its significance. — Genetica 5: 225—272.
22. DOBZHANSKY, TH. 1934. Studies on hybrid sterility. I. Spermatogenesis in pure and hybrid *Drosophila pseudoobscura*. — Ztschr. f. Zellf. u. mikr. Anat. 21: 169—223.
23. EHRENBERG, L. 1945. The shape of the spindle at metaphase is conditioned by the shape of its molecules. — Hereditas XXXI: 240.
24. EHRENBERG, L. and ÖSTERGREN, G. 1942. Experimental studies on nuclear and cell division. — Bot. Not. 1942: 203—206.
25. FRANKEL, O. H. 1937. Inversions in *Fritillaria*. — Journ. of Genetics 34: 447—462.
26. FRANKHAUSER, G. 1934. Cytological studies on egg fragments of the salamander *Triton*. IV. The cleavage of egg fragments without the egg nucleus. — Journ. Exp. Zool. 67: 351—391.
27. GOODSPEED, T. H. 1946. Meiotic prophase phenomena in species and interspecific hybrids of *Nicotiana*. — Journ. Arnold Arbor. 27: 453—469.
28. GUSTAFSSON, Å. 1948. Polyploidy, life-form and vegetative reproduction. — Hereditas XXXIV: 1—12.
29. KAGAWA, F. and CHIZAKI, Y. 1934. Cytological studies on the genus hybrids among *Triticum*, *Secale* and *Aegilops* and the species hybrids in *Aegilops*. — Jap. Journ. Bot. 7: 1—32.
30. KOLLER, P. C. 1943. Origin of malignant tumour cells. — Nature 151: 244—246.
31. — 1947. The experimental modification of nucleic acid systems in the cell. — Symp. Soc. Exp. Biol. 1. Nucleic acid: 270—290.
32. LEVAN, A. 1938. The effect of colchicine on root mitoses in *Allium*. — Hereditas XXIV: 471—486.
33. LEVINE, M. 1931. Studies in the cytology of cancer. — Amer. Journ. Cancer 15: 144—211 and 1410—1494.

34. LIMA DE FARIA, A. 1947. Disturbances in microspore cytology of *Anthoxanthum*. — Hereditas XXXIII: 539—551.
35. LOVE, R. M. 1936. Occurrence of haploid pollen mother cells in a *vulgare* wheat. — Nature 138: 589—590.
36. MATHER, K. 1945. The genetical activity of heterochromatin. — Proc. Roy. Soc. London B 132: 308—332.
37. MC KNIGHT, R. H. 1937. Haploid ovarian tissue in *Drosophila*. — Proc. nat. Acad. Sci. Wash. 23: 440—443.
38. METZ, C. W. 1933. Monocentric mitosis with segregation of chromosomes in *Sciara* and its bearing on the mechanism of mitosis. — Biol. Bull. 64: 333—347.
39. MEURMAN, O. 1928. Cytological studies in the genus *Ribes* L. — Hereditas XI: 289—356.
40. NEWTON, W. C. F. and PELLEW, C. 1929. *Primula kewensis* and its derivatives. — Journ. of Genetics 20: 405—467.
41. PARASARATHY, N. 1938. Cytogenetical studies of some x-ray derivatives in rice (*Oryza sativa* L.). — Journ. of Genetics 37: 1—40.
42. SASS, J. E. 1938. Abnormal mitosis in seedlings of some Gramineae following seed treatment. — Amer. J. Bot. 25: 624—627.
43. SAX, K. 1935. The effect of temperature on nuclear differentiation in microspore development. — Journ. Arnold Arbor. 16: 352—356.
44. — 1937. Chromosome behavior and nucleal development in *Tradescantia*. — Genetics 22: 523—533.
45. SCHRADER, F. 1944. Mitosis. The movements of chromosomes in cell division. — New York.
46. — 1947. Data contributing to an analysis of metaphase mechanics. — Chromosoma 3: 22—47.
47. SMITH, LUTHER, 1942. Cytogenetics of a factor for multiploid sporocytes in barley. — Amer. J. Bot. 29: 451—456.
48. STEBBINS, G. L. JR. 1938. Cytological characteristics associated with the different growth habits in Dicotyledons. — Amer. J. Bot. 25: 189—198.
49. — 1947. Types of polyploids: Their classification and significance. — Adv. in Genetics 1: 403—429.
50. SWANSON, C. P. 1942. Meiotic coiling in *Tradescantia*. — Bot. Gaz. 103: 457—474.
51. SWANSON, C. P. and NELSON, R. 1942. Spindle abnormalities in *Mentha*. — Bot. Gaz. 104: 273—280.
52. SVÄRDSON, G. 1945. Chromosome studies in Salmonidae. — Rep. Swedish State Inst. of Fresh-Water Fishery Res. 23: 1—151.
53. UPCOTT, M. 1937. Timing unbalance at meiosis in the pollen-sterile *Lathyrus odoratus*. — Cytologia, FUJII Jub. Vol.: 229—310.
54. — 1939 a. The nature of tetraploidy in *Primula kewensis*. — Journ. of Genetics 39: 79—100.
55. — 1939 b. The external mechanics of the chromosomes. VII. Abnormal mitosis in pollen grain. — Chromosoma 1: 178—190.
56. VAARAMA, A. 1947. Morphological and cytological studies on colchicine-induced tetraploid *Ribes nigrum*. — Acta Agr. Fenn. 67, No. 2: 55—92.
57. — 1948. Meiosis and polyploid characters in the tetraploid apple variety Hibernal. — Hereditas XXXIV: 147—160.

58. WALD, H. 1936. Cytologic studies on the abnormal development of the claret mutant type of *Drosophila simulans*. — Genetics 21: 264—281.
59. ÖSTERGREN, G. 1944. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. — Hereditas XXX: 429—467.
60. — 1945. Equilibrium of trivalents and the mechanism of chromosome movements. — Hereditas XXXI: 498.
61. ÖSTERGREN, G. and PRAKKEN, R. 1946. Behaviour on the spindle of the actively mobile chromosome ends of rye. — Hereditas XXXII: 473—494.

CONTENTS.

I. Introduction 136
II. Material and methods 137
III. Results 138
1. Normal conditions 138
2. Spindle abnormalities 139
3. Timing abnormalities in the development of sister spindles 141
4. Variation of somatic chromosome numbers 143
IV. Discussion 147
1. Evidence from the frequency of different chromosome numbers 147
2. Causes and results of the split spindle formation 151
Summary 158
Literature cited 159

STUDIES ON X-RAY MUTATIONS IN THE WHITE AND FORKED LOCI OF DROSOPHILA MELANOGASTER

I. A STATISTICAL ANALYSIS OF MUTATION FREQUENCIES

BY *G. BONNIER* AND *K. G. LÜNING*
INSTITUTE OF GENETICS, UNIVERSITY OF STOCKHOLM, SWEDEN

I. INTRODUCTION.

ALREADY during the earlier days of radiation genetics it was found that the mutation frequency was linearily related to dosage (HANSON and HEYS, 1929, 1932; OLIVER, 1930, 1932; TIMOFÉEFF-RESSOVSKY, 1931, 1934. For further literature, see TIMOFÉEFF-RESSOVSKY, 1937; MULLER, 1940, 1941; LEA, 1946). This finding has formed one of the important bases of the »target theory», according to which each mutational effect is due to one hit within or at a short distance from the target and producing one single ionization. If true, this very useful and far-reaching theory necessitates a strict proportionality between number of hits and dose, and thus, with regard to the rising probability at rising doses of several hits within a single target, a strictly exponential shape of the mutation frequency curve. At lower doses, therefore, even small deviations from linearity would, if these deviations were real, tend to diminish the range of validity of the target theory.

Now, the *Drosophila* experiments in which the linearity question has been investigated have been performed by the use of a great number of different doses, the majority of which have been rather high (more than 3000 *r*; a notable exception from this is, however, the recent work by SPENCER and STERN, 1948, which will be discussed in the second part of the present study), thus necessarily including a large proportion of multiple-hit cases. Most of these investigations have furthermore been based on studies of lethal mutations. As is well known, this has the advantage of minimizing the bad effects of the differential visual acumen of the observers. However, the impossibility of differentiating between true lethals and semilethals, and the fact that there are several hundreds of targets per chromosome ready for

lethal mutations, may from certain points of view be considered as causes for hesitation in the use of lethals in a study of the linear relationship. In the earlier studies, finally, the statistical treatment of the data have mainly been based on the use of standard errors. As pointed out by STEVENS (1942), this is certainly incorrect, as the distribution of the mutation frequencies within doses is very far from normal. To aid investigators to correct their estimates of error he has computed a useful table based on the Poisson distributions for a number of small averages. But, as it is *a priori* uncertain whether or not the variations within doses are truly random, these error-estimates might be misleading even after STEVEN's corrections are made.

It has, therefore, seemed desirable to the senior author to undertake with *Drosophila* X-ray experiments fulfilling the following requirements: (1) a number of reasonably low doses should be used; (2) the observations should involve few single loci in which visible mutations are as easily detectable as possible; (3) the classification of the flies forming the basis of the frequency studies should be performed in such a way as to make it possible to estimate the variation within doses directly from the experimental results themselves; (4) the experiments should be made on such a scale as to make a critical statistical examination possible.

The original plans of the present study were made up several years ago, but it has not been possible to put them into effect until now. The investigation is divided into two parts. The present paper, which forms the first part, contains a presentation of material and methods and a statistical analysis of the variation within doses as well as of the linearity problem. Thus, the contents of the present paper will mainly be of a statistical nature. In the second part, which will be published shortly, a survey will be given of a number of special cases of mutations and of their possible significance. In this latter part the results of both parts will be discussed.

Acknowledgements. — The authors are deeply indebted to the Swedish Natural Science Research Council, which has supported this investigation. We wish also to thank all those who have helped us in our work. We owe our great gratitude to Miss G. CEDERWALL, Mrs. S. HENNINGS, Mr. L. EKEBLAD and Mr. J. HAMMARÉN for their enthusiastic and painstaking help in counting and classifying the flies, to Mrs. S. REIJHELL for her technical assistance and to Drs. A. M. PERJE, B. RASMUSON and M. RASMUSON for aiding us in taking care of mutant stocks. We are very much indebted to Professor R. SIEVERT and Dr. A. FORS-

BERG for their courtesy in facilitating the irradiation work and to Miss V. LINDAHL for her assistance in this work. We wish to thank Professor H. CRAMÉR for his many valuable suggestions concerning statistical questions and for the appropriate methods he has put at our disposal. We are deeply indebted to Professor T. CASPERSSON, who has given our laboratory a refuge in its temporary institutional difficulties. Without his generosity our work could not have been carried out.

II. MATERIAL AND METHODS.

The irradiations were performed at the Radiophysical Institute with the use of a Siemens Stabilivolt apparatus of 155 kilovolt with 1 mm Al. Exposures have, as a rule, been made twice weekly.

A wild type stock maintained in our laboratory for several years has been the source of wild males for irradiation. Two different types of females have been mated to the X-rayed males. During the earlier part of the work we used females from a stock homozygous for white and forked, but this stock was later replaced by one homozygous for yellow, white, forked and Beadex. The genes yellow and Beadex have ben introduced as marker genes, the frequency studies being confined to the white and forked loci.

During the course of the work some small modifications have been made in the routine. The different steps of the work may, however, be satisfactorily described as follows. The stock from which the females were taken, the »female stock», has been maintained by collecting eggs for one to a few days from mass cultures of about 250 males and 250 females per bottle. During the first 3 to 4 days of hatching of the progeny, virgin females were collected twice daily, and these females were stored without males for periods as long as 7 days. The stock from which wild males were collected for irradiation, the »male stock», has been maintained in the same way as the »female stock», but collections of male progeny were made only once every three days. These males were stored for one day without females, after which they were X-rayed. Their age at raying thus ranged as a rule from 2 to not more than 5 days. In a very few instances they may have been somewhat older. Immediately after being X-rayed the males were mated to the stored virgin females from the »female stock». In each culture bottle about 150 females and 100 to 150 males were left for 2 to 3 days. Thereafter, the females were transferred to new culture bottles, usually 2 to 3 females per bottle, without males. These bottles were emptied after

13 to 17 days depending on various circumstances (holidays, *etc.*) and the progeny classified. These bottles were then discarded, so that the classification of the progeny has only been made on one single occasion for each bottle. The incubators were run at 25° C.

Apart from a number of different doses which were tried during a pre-experimental stage of the present studies (including 2908 *r*; *cf.* below), 4 doses were used, and in addition to these 3 kinds of controls were arranged. The 4 doses were planned to be 800 *r* (series VII), 1600 *r* (series V), 2400 *r* (series III) and 3200 *r* (series I). When the experiments began, an intensity check was made by means of Sievert's ionization chamber, and the distance between animals and source chosen for the 800 *r* series was such that these 800 *r* should take a time of 1,5 minutes. The other 3 doses were then obtained by irradiating the males at the same distance for 3, 4,5, and 6 minutes, respectively. During the course of the experiment 36 more measurements were made of the intensity at the distance used, and the average was found to be $484{,}75 \pm 4{,}24$ *r* per minute. The 4 doses were thus somewhat lower than planned, being 727 *r*, 1454 *r*, 2181 *r* and 2908 *r*, respectively.

The 3 series of controls were as follows. One of the series, series K, was a normal control, *i. e.* no X-raying was made. The 2 other control series planned to be a kind of extension of the true control, series K, were irradiated at the very low dose of about 10 *r*. In one of these series, series XI, the same distance between source and males as before was used and exposure of one second was attempted. This would mean that the actual dose was $8 \pm 0{,}71$ *r*. In the other series, series X, a much longer distance was chosen and 2 intensity measurements gave 8,0 and 8,7 r/min. The time used in this series was 2 minutes, so the dose administered was between 16 and 17 *r*.

For practical reasons it was impossible to give more than one single dose on any one occasion, but the doses were usually alternated. Table 1 shows the total number of times and the dates of the first and the last start of the cultures from which the progeny was classified. As may be seen, the three series VII, V and III, the 3 lowest doses, were alternated. The highest dose, series I, was not used until after the experiments with the lower doses had come to an end, the reason being that in the pre-experimental trials the experiments began with this series I, chiefly made in order to gain experience. The registering of the results and the managing of the stocks were not done in the manner later adopted. Thus these early results were difficult to compare with the results of later series and it was decided to discard the early part

TABLE 1. *Survey of the material.*

Series	Dose *r*	Number of times the dose was used	Dates of first and last start of culture from which progeny was counted [1]	Type of homozygous females to which irradiated males were mated	Total offspring		
					females and gynandromorphs	males	Total
VII a	727	20	Feb. 20—June 15	*w f*	371338	330964	702302
VII b	727	6	July 13—Aug. 6	*y w f Bx*	30667	22054	52721
Total VII	727	26	Feb. 20—Aug. 6		402005	353018	755023
V a	1454	6	Feb. 17—May 10	*w f*	113563	105616	219179
V b	1454	12	July 2—Oct. 1	*y w f Bx*	130979	102658	233637
Total V	1454	18	Feb. 17—Oct. 1		244542	208274	452816
III a	2181	6	Feb. 3—May 15	*w f*	74945	70633	145578
III b	2181	10	June 16—Nov. 26	*y w f Bx*	98001	79404	177405
Total III	2181	16	Feb. 3—Nov. 26		172946	150037	322983
I	2908	6	Oct. 8—Nov. 23	*y w f Bx*	92409	79855	172264
X	16	5	Dec. 3—Jan. 14	*y w f Bx*	62343	49000	111343
XI	8	6	Dec. 21—Jan. 7	*y w f Bx*	88874	70024	158898
K	0	9	Oct. 5—Nov. 30	*y w f Bx*	153579	119114	272693
Grand Total					1216698	1029322	2246020

[1] The dates of the ending of the experiments X and XI refer to 1949, all other dates to 1948.

of series I and to make a new series I. The 3 control series were likewise made at the end of the experiment. The reason here was that they were not planned from the beginning, it being the results of the earlier work that showed the necessity of having controls. Table 1 also gives the total number of flies classified within the different series.

III. THE CLASSIFICATION OF THE PROGENY OF X-RAYED MALES, AND SOURCES OF ERROR IN THIS CLASSIFICATION.

The chief interest lies in the nature of the female (and gynandromorphous) progeny. During the earlier part of the experiments all kind of females which were non-wild with regard to eye-colour or bristle

TABLE 2. *Frequencies of mutations.*

(Figures in brackets show the number of mutants which have been progeny tested.)

Series	Dose *r*	Number on which relative frequencies are based	white		fractional white	white + fractional white		forked		mosaics due to elimination of paternal *X*-chromosome	
			abs. number	per 10^4	abs. number	abs. number	per 10^4	abs. number	per 10^4	abs. number	per 10^4
VII	727	385283 forked 402005 others	40 (28)	0,9950	10 (4)	50 (32)	1,2438	38 (26)	0,9863	37 (7)	0,9204
V	1454	244542	36 (22)	1,4721	6 (4)	42 (26)	1,7175	26 (19)	1,0632	23 (3)	0,9405
III	2181	162064 white, forked 172946 others	28 (22)	1,7277	9 (2)	37 (24)	2,2830	33 (25)	2,0362	37 (6)	2,1394
I	2908	67002 forked 92409 others	22 (10)	2,3807	3 (3)	25 (13)	2,7054	14 (11)	2,0895	29 (2)	3,1382
X	16	62343	0		1 (1)	1 (1)		0		5 (0)	0,8020
XI	8	88874	0		1 (1)	1 (1)		0		9 (0)	1,0127
K	0	153579	0		0	0		1 (1)		9 (1)	0,5860
K+X+XI		304796	0		2 (2)	2 (2)		1 (1)		23 (1)	0,7546

characters were mated to brothers. A great number of gynandromorphs were also mated to brothers. Later, these matings were confined to a limited number of aberrational types. Of course, not all mated females gave progeny. Many were decidedly sterile, in other cases the females were lost through accidents of diverse kinds, and as a result of a few severe mould attacks the progeny was, in some cases, prevented from

developing to the imaginal stage. In Table 2, which shows the number of aberrational types in the different series, the figures within brackets indicate the number of these types which were actually progeny-tested. The results of these progeny-tests will be discussed in the second part of the present study.

The following different aberrations were recorded and classified: full mutants white; full mutants forked; mutant eye-colours not white, recorded as »eosin-like», »coral-like», *etc.* and as »dark eyes»; variegated eyes; fractional white, *i. e.* animals having only a part of the eyes white but not showing any signs of the other mutant characters; fractional forked, *i. e.* animals having only a part of the body forked but showing no signs of the other mutant characters; other mosaics and gynandromorphs.

There are always some possibilities that the classifications have been erroneously made. The reasons for this in the case of the different aberrational types are as follows: It is quite impossible that a *full white* should escape attention, but an animal with white eyes might, instead of being a mutation from wild to white, be the result of a chromosome elimination in only the eyes together with, perhaps, a few surrounding hairs. These hairs would then, if the elimination was not accompanied by a chromosome breakage, be forked or yellow forked, depending upon the mothers' genetic constitution (*cf.* p. 165). However, a very few such hairs might escape attention, though this is not probable. It is also quite impossible that a *full forked* should escape unnoticed, but it might be a fractional, or a mosaic due to chromosome elimination, as the forked character (with the forked gene which we have used) does not show very well on certain parts of the body, *e. g.* abdomen and legs. For the same reasons animals which have been registered as *fractional white* may, instead of being fractionals, be mosaics due to chromosome elimination. It is also conceivable that such fractionals might escape attention if the white area occupies only a very few facets. In the case of *fractional forked* several mistakes may be made. If there are only a few bristles showing a more or less forked-like type, these may be overlooked or, if not overlooked, the aberration may be due to something other than a mutation in the f^+ gene. A very great number of females have shown one or two aberrant bristles, usually the scutellars, and several hundreds of these have been mated to brothers but their progeny has not shown any sign of forked phenotype. Only in one single case was the whole progeny forked — it will be discussed in the second part of this study. A fractional forked in which

the forked area only occupies some of the abdominal segments could also easily be overlooked. With regard to *non-white* eye-colour aberrations, confusions between white alleles and variegated (mottled) white may be made, and »*dark eyes*» may also be the result of translocations of different kinds. Finally, with regard to *gynandromorphs* and other *mosaics* due to chromosome elimination, these may be overlooked if the aberrant area is very limited and especially if it is situated on abdominal segments without affecting the genetalia, *e. g.*, if patches of forked and yellow are hidden under the wings of etherized animals. Further, patches situated on sternites always show up badly. Mosaics due to elimination of the maternal untreated *X*-chromosome would be detected only if the animals were typical gynandromorphs as shown by their genitals, rotated abdomen, and so forth. As a consequence of the greater difficulty to detect mosaics due to maternal than to paternal chromosome elimination, many more of the latter than of the former kind have actually been observed. Mosaics due to maternal elimination are, accordingly, not included in the present study.

That part of the present study in which mutational frequencies at different doses are compared must be based on as reliable data as possible. We are convinced that the errors which may have been made in classifying cases as full mutants white or forked must be so extremely few as to have no influence on the results. To nearly the same extent this may also be said of fractional whites. However, as they are rather few, they have been pooled with the full whites in the analyses. The frequencies of gynandromorphs and other mosaics due to chromosome elimination are less reliable. It is also practically certain that the higher training of our co-workers' capacities for detecting aberrant types during the later part of the work has made the frequencies of these mosaics relatively too high in series I, K, X, XI (see Table 1) in comparison with the frequencies of the series VII, V, III. They are accordingly not included in the statistical analyses of the linearity problem. The classifications of kinds of aberrant types other than those just mentioned are less reliable and are therefore not included in any of our analyses. Several of these will, however, be taken under consideration in the second part of the present study. The total number of the 4 types of aberrants in question are shown for the different doses in Table 2 (in which the series VII a and VII b, V a and V b, III a and III b, Table 1, have been pooled). There is one thing in this table which necessitates a special explanation. As seen, the total number of flies on which the mutational frequencies are computed are not equal for

all 4 types of aberrations. This is due to circumstances of the following kind. It once happened, for instance, that a forked male was found in the wild stock from which the males for X-raying were collected. This male was either due to contamination or to a spontaneous mutation within the wild stock. The whole lots from two consecutive radiations within series I, which were then under way, were therefore discarded as far as they concerned forked mutations, but not with regard to other aberrations. The other discrepancies associated with the total number of flies on which the different mutation frequencies are based are due to more or less similar causes.

IV. THE REGISTRATION OF THE RESULTS AND THEIR STATISTICAL EVALUATION.

As was pointed out in the introduction, one of the aims of our studies was of a statistical nature, *viz.* on the one hand to be able to estimate from the data themselves the amount of variation within doses, and, on the other hand, to be able to judge to what extent uncontrolled environmental influences may affect the frequencies of mutation. In order to make this possible the classification of female progeny (including gynandromorphs) of irradiated males was made in lots of 1000 individuals, and for each such lot of 1000 individuals the number and the nature of the different mutants were recorded. To facilitate this work each sheet used for the counting of the flies was limited to exactly 1000 flies. After each new instance of irradiation the flies were grouped in new lots of 1000, which means that if the females (among which the gynandromorphs are included here and below) which emerged after one irradiation did not amount to an exact multiple of 1000 the surplus was treated as a remainder. Furthermore, since usually 3 to 5 co-workers were active with the countings and classifications, each one could get a remainder.

The procedure that was followed for the final registration — which thus constituted no source of trouble to the observers themselves — may conveniently be explained by an example. The sets of cultures made up after the three first radiations in series VII were started on February 20, 24 and 27, respectively. Five co-workers, A, B, C, D, E, did the countings. In the set of the 20th of February 20 full thousands of females were counted. The first thousand to be counted from the set of the 24th of February thus got the ordinal number 21. In this set A counted 5 full thousands and a remainder of 811 (*i. e.* altogether

5811). A's full thousands thus got the ordinal numbers 21—25. Within number 22 there occurred a forked and within number 25 a gynandromorph. B counted 5 full thousands, numbers 26—30 and a remainder of 431. Within number 27 there occurred a forked, and within the first hundreds of the remainder a white. C counted 6 full thousands, numbers 31—36 and within number 35 there occurred a forked. When C had counted the full 6 thousands there were left a few flies in his last bottle, and these were taken over by one of the other co-workers, so C got in this way no remainder. D counted 5 full thousands, numbers 37—41, and had a remainder of 113. Within number 39 there was a white. E counted 4 full thousands, numbers 42—45 and a remainder of 46, with a gynandromorph within number 42. The full thousands from the sets of 24th of February thus ranged from 21 to 45. But the remainders, 811 + 431 + 113 + 46, gave together 1401, thus still one full thousand number 46 and a *final* remainder from this set of 401 females. As mentioned above, B found a white within the first hundreds of his remainder. This white was accordingly reckoned as belonging to the thousand with the ordinal number 46. But, if it had been C who had found this white, it would have fallen out of this thousand and accordingly been registered within the final remainder of the set of February 24th. The set of February 27th began, consequently, with the thousand-number 47 counted by co-worker A.

Our first task is now to find out whether the different kinds of aberrations are distributed over the 1000-lots according to the Poisson distribution. When we gave the total and relative frequencies of mutants in Table 2, the whole material was used, and this will also be the case in the analysis of linearity. But for our present task we shall, of course, not include those aberrants — rather few in number — which have fallen within the *final* remainders of each separate set. One more cause of discrepancy between total numbers of aberrants for the present Poisson test and the actual total number is the following. The relative frequency of mutants is, for instance, in the case of series VII (dose 727 *r*) so low that there should be practically no cases of 1000-lots with more than one aberrant of any kind. Therefore the lots of 1000 have been combined — always in ordinal sequence — in this case into groups of 4, *i. e.* each lot has been made to consist of 4000 instead of 1000 flies. Now, however, the total number of 1000-lots available for white is 389 in series VII. This makes 97 lots of 4000 and one thousand left. Had any aberrant fly fallen within this last thousand it should accordingly not be included in the Poisson test. In series V

(dose 1454 *r*) the lots of 1000 have for similar reasons been grouped into lots of 3000, in series III (dose 2181 *r*) into lots of 2000, whereas in series I (dose 2908 *r*) the lots of 1000 have remained ungrouped. Table 3 shows the total number of aberrants which for these two quite random causes have been left out of the Poisson tests.

TABLE 3. *Number of mutants falling within remainders and therefore not included in the Poisson tests.*

Mutant	Series	Number in groups	remainders in	
			radiation sets	last thousand off grouping
white	VII	4000	—	—
	V	3000	2	2
	III	2000	—	1
	I	1000	—	—
fractional white	VII	4000	—	—
	V	3000	—	—
	III	2000	1	—
	I	1000	—	—
forked	VII	4000	1	—
	V	3000	2	—
	III	2000	2	1
	I	1000	—	—
mosaics due to elimination of paternal *X*-chromosome	VII	4000	—	—
	V	3000	2	—
	III	2000	—	—
	I	1000	—	—

The expected numbers of groups with 0, 1, 2 ... aberrants were calculated from the frequencies of the whole material as given in Table 2. Table 4 shows the result. (The expected numbers in the rightmost class are computed directly so that the total expected tallies with the total observed). It is now to be remembered that the χ^2 test exaggerates the discrepancy between »observed» and »expected» when the expected number is small, 5 or less. But if, in view of this fact, we had pooled the classes, there would in most cases have remained only

TABLE 4. *Poisson test.*

(O = observed, E = expected No. of groups.)

Mutants	Series		Number of groups with the indicated number of mutants					χ^2	Degrees of freedom	*P*
			0	1	2	3	4			
white	VII	O	64	27	5	1		0,15	3	0,99—0,98
		E	65,15	25,93	5,16	0,76				
	V	O	51	23	3	1				
		E	50,15	22,15	4,89	0,81		0,82	3	0,90—0,80
	III	O	54	19	4					
		E	54,50	18,83	3,67			0,04	2	0,99—0,98
	I	O	72	13	3	1				
		E	70,15	16,70	1,99	0,16		5,79	3	0,20—0,10
	Total							6,80	11	0,90—0,80
white + fractional white	VII	O	59	28	8	2				
		E	58,98	29,34	7,30	1,38		0,41	3	0,95—0,90
	V	O	47	26	3	2				
		E	46,59	24,01	6,18	1,22		2,30	3	0,70—0,50
	III	O	48	24	4	1				
		E	48,77	22,27	5,08	0,88		0,39	3	0,95—0,90
	I	O	69	16	3	1				
		E	67,91	18,37	2,48	0,24		2,84	3	0,50—0,30
	Total							5,94	12	0,95—0,90
forked	VII	O	63	25	3	2				
		E	62,68	24,73	4,88	0,71		3,07	3	0,50—0,30
	V	O	57	18	3	—				
		E	56,70	18,09	2,88	0,33		0,34	3	0,98—0,95
	III	O	50	25	1	1				
		E	51,24	20,87	4,25	0,64		3,54	3	0,50—0,30
	I	O	51	14	—					
		E	52,74	11,02	1,24			2,10	2	0,50—0,30
	Total							9,05	11	0,70—0,50
mosaics due to elimination of paternal *X*-chromosome	VII	O	67	23	7	—				
		E	67,12	24,71	4,55	0,62		2,06	3	0,50—0,70
	V	O	63	10	4	1				
		E	58,83	16,60	2,34	0,23		6,68	3	0,10—0,05
	III	O	55	19	7	—	1			
		E	53,46	22,87	4,89	0,78		1,67	3	0,50—0,70
	I	O	68	14	6	1				
		E	65,03	20,41	3,20	0,36		5,74	3	0,20—0,10
	Total							16,15	12	0,20—0,10

classes for 0 or more than 0 aberrants per group and the figures in Table 4 would not have given us the impression of such very good agreement between »expected» and »observed». But the fact that we have not pooled the classes — except in the single case of one group in series III giving not less than 4 mosaics — means that the agreements are closer than the P values indicate. We may therefore conclude that the observed distributions are in exceedingly good agreement with the Poisson law. Only the mosaics do not show such a good fit, but if we had pooled to only two classes in series V and I we should have found for these series the two P values $0{,}3$—$0{,}2$ and $0{,}5$—$0{,}3$, respectively; and for the total of mosaics we should have found a P value of $0{,}8$—$0{,}7$.

The very good agreement with the Poisson distribution proves, we believe, that the variations in mutation frequencies between the different instances of radiation due to non-intentional and unknown causes have been effectively cancelled by the repetition of each dose several times. It may, however, be asked whether there is any noteworthy variation within doses between different instances of radiation, apart from that due to random causes. In order to get an answer to this question we have made a χ^2 test of homogeneity in the following way.

Within each dose we have selected all those sets, *i. e.* all those individual instances of radiation, which have given at least 4 groups of female progeny. That is to say, we have selected all sets which in series VII have given at least 4×4000 individuals in the progeny; in series V at least 4×3000; in series III at least 4×2000; and in series I at least 4×1000 individuals in the female progeny. In series VII within the set dated 24th of February there were 26 lots of 1000, or 6 groups of $4000 + 2$ lots of 1000. For the present analysis, then, only the first 24 lots of 1000 have been used, leaving out of consideration what may have happened within the last two lots of 1000 — and, of course, also what may have happened within the remainder of this set. In this way it is possible to tabulate in how many groups *within* each such set there have been observed 0, 1, 2 . . . aberrants of any kind, and to make a χ^2 analysis of the comparison with expectation. This expectation has been based, as before, on the data of Table 2. As the expected numbers within the different classes must now be very low, the χ^2 test strongly exaggerates the discrepancy between observation and expectation. We have accordingly pooled, so that there only remains the classes 0 and more than 0. Table 5 gives the results, which show that the heterogeneity

is, at any rate, not very pronounced. It is only in the case of white, series III and I — and as a consequence also in the case of total white — that P is less than 0,05. But in contradistinction to this, Table 4 showed a very good agreement between observation and expectation for white.

TABLE 5. *Heterogeneity test.*

Mutant	Series	χ^2	Degrees of freedom	P
white	VII	15,74	12	0,30—0,20
	V	8,74	10	0,70—0,50
	III	19,96	11	0,05—0,02
	I	14,23	6	0,05—0,02
	Total	58,67	39	0,05—0,02
white + fractional white	VII	13,87	12	0,50—0,30
	V	6,48	10	0,80—0,70
	III	19,33	11	0,10—0,05
	I	10,22	6	0,20—0.10
	Total	49,90	39	0,25—0,20
forked	VII	12,36	11	0,50—0,30
	V	9,00	10	0,70—0,50
	III	12,44	11	0,50 - 0,30
	I	1,28	4	0,90—0,80
	Total	35,08	36	0,96
mosaics due to elimination of paternal *X*-chromosome	VII	5,83	12	0,95—0,90
	V	9,70	10	0,50—0,30
	III	23,95	12	0,05—0,02
	I	6,92	6	0,50—0,30
	Total	46,40	40	0,50—0,40

In an experiment to determine mutation frequencies after X-ray treatment, conducted in the main as ours, it seems safe to conclude: (1) that the variation in mutation frequency between different instances of irradiation due to unintentional and unknown causes is not very much larger than would be expected from random causes and (2) that by repeating the irradiations several times the variation between the different instances of irradiation due to non-random causes is effectively controlled. From these considerations we may thus also conclude that the frequencies of mutations found by us for different doses are »true» expressions of the mutation rates for our stocks under the general con-

ditions governing our experiments. This means that our observed frequencies are well suited for a closer statistical analysis of the relation between dose and mutation frequency.

V. THE LINEAR DOSE-MUTATION FREQUENCY RELATION.

The target theory requires a strictly linear relation between dose and mutation frequency at low doses. There are, however, two well known causes of deviation from linearity. The first of these is due to the so-called »saturation» effect, *i. e.*, two or more hits within one target will be observed as one single aberrant. This leads to a deviation from linearity, giving the expected curve an exponential shape. However, as the highest of the doses used in the present experiments is as low as 2908 *r*, and as the targets in our case are single loci, this cause of deviation cannot be expected to have any appreciable effect.

The other cause that may influence the dose-mutation frequency relation so as to make it non-linear or change the slope of the linear relation is the differential mortality at different doses. That the preimaginal mortality increases with rising dose has been demonstrated many times. From this fact it is possible to argue in two different ways and arrive at two opposite conclusions. In the first place, we may suppose that the kinds of mutants we are studying are weakened relatively more than are the wild daughters of irradiated males. If that were so, a relatively higher proportion of mutants would die before hatching at higher than at lower doses, and the slope of the true regression of mutation rate on dosage would be steeper than the regression estimated from the actual data. A relatively higher degree of weakening of the mutants at higher than at lower doses could be expected for two reasons. We can either assume that at higher doses several hits occur within the target with higher frequency than at lower doses — making at the same time the plausible assumption that more hits damage a target more than fewer hits. This supposition is, however, the same as that leading to the »saturation» effect, which, as said above, may be neglected. Or, it could be assumed that the mutant occurred after hits, not only within the wild gene itself, but also outside the gene and even at rather long distances. If so, the size of the target is actually larger than that of the wild gene, and the mutants could be the result of, sometimes one, sometimes two and sometimes more hits (with or without breaks leading to recombinations of diverse

kinds). If this were true, a relatively higher number of mutants would escape attention at higher doses; but it would at the same time also be probable that a certain number of the multiple hit cases would be viable, with the consequence of a mutation frequency curve rising at a higher power of the dose than the first. This is contradictory to several earlier results and also to our own experiments. (This does not, of course, exclude the possibility of mutations occurring in single cases together with translocations, even at the low doses of our experiments).

The opposite result would be obtained if we, as is usually done, assume that each mutation is the result of one single hit, and that the damaging effect of this hit is independent of the dose (though different hits have different damaging effects). Then the pre-imaginal mortality of the mutants would be relatively higher in comparison with that of the wild females at lower than at higher doses, and a relatively larger number of mutants would escape our attention at lower than at higher doses. The consequence would be that the true regression would be less steep than that estimated from the observed data.

From the above considerations it thus seems more probable that the observed regressions are more rather than less steep than the true regressions. It is, however, impossible to deduce the extent to which the observed line might be too steep. At any rate, it seems safe to suppose that, within limits of error, the observed regression coefficients are not too small. Also, from the points of view now discussed, we feel justified in using the observed mutation frequencies, Table 2, in a critical statistical analysis, and also in assuming that any errors introduced in so doing emanate almost entirely from random causes.

As mentioned above (p. 166), at the outset we used 4 doses which were planned to be 800 *r*, 1600 *r*, 2400 *r*, 3200 *r* (series VII, V, III, I; Table 2). By a number of intensity measurements it was later found that actually the doses were lower, being 727 *r*, 1454 *r*, 2181 *r*, 2908 *r* (Tables 1 and 2). Their relative magnitudes were, however, still proportioned as 1 : 2 : 3 : 4, and in the statistical treatment, now to follow, the doses will be expressed by these four integers. The dose, expressed in this new unit, will be denoted by x, thus $x = 727\ r$.

From the time when a rather large mass of data had been accumulated, the regressions and their linearities were studied on several occasions. It was always observed that if the regressions computed from the 4 doses, $x = 1, 2, 3, 4$, were extrapolated to the dose $x = 0$, its intersection with the ordinate axis was rather high — and higher than

could be expected to correspond to spontaneous mutation rates. It was therefore, as well as for some other reasons, decided to make a set of control experiments, series K (Tables 1 and 2). (The reason series I was repeated at the same time and its older data discarded has been mentioned above, p. 166.) Within series K we found no white or fractional white and only one forked (Table 2). We therefore decided to extend the control series with a new series given an extremely low dose, in order to see whether this tallied with the spontaneous rate or

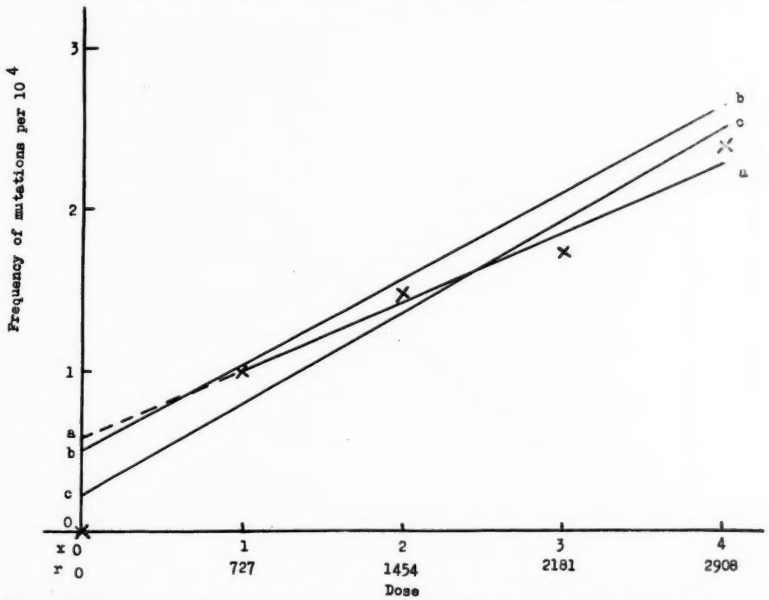


Fig. 1. Regression lines *a*, *b* and *c* (*cf.* text) corresponding to mutations from w^+ to *w*. Crosses mark observed mutation frequencies.

with that expected from the regression of the X-ray experiments. So we started series X (Table 2). As mentioned above (p. 166), the intensity in this series was lower than that in our other X-ray series. But as we seem to know very little about the influence of intensity at such very low doses and moderate wave lengths (most experiments on that problem at low doses have been made with gamma-rays; see MULLER, 1940; RAYCHAUDHURI, 1939, 1944; CASPARI and STERN, 1948), series XI (Table 2) was started.

In the statistical treatment below we have for each of the 3 muta-

tional combinations investigated — white, white + fractional white and forked — computed the ordinary regression lines based on the mutation rates (from Table 2) at the doses $x = 1, 2, 3, 4$. These lines are named lines *a* (Figs. 1—3). The tests of linearity of these regressions ought not to be made in the ordinary way (FISHER, 1936, Ex. 42, p. 257) by comparing the residual variance of the means with the average variance within doses. The reasons are twofold. On the one hand, as we have seen, the distribution within doses is of the Poisson type and

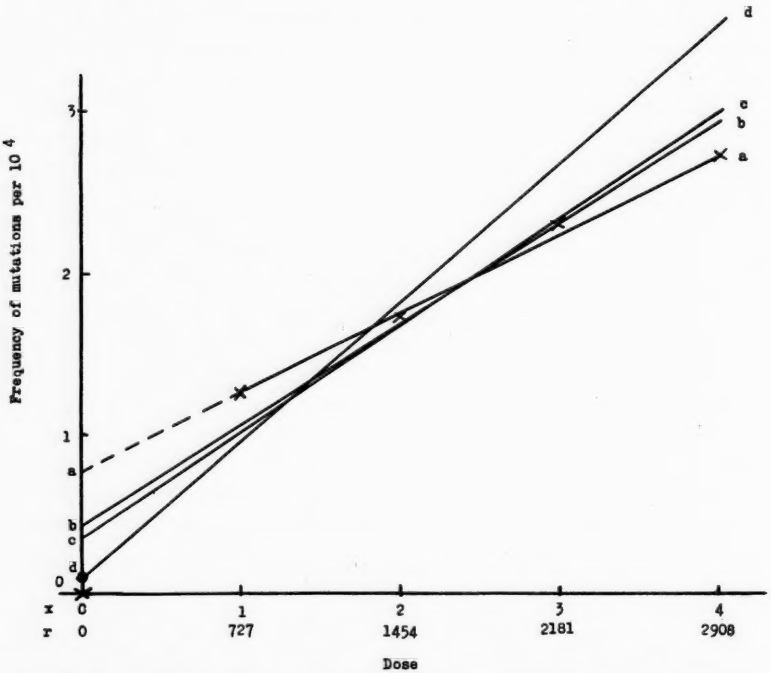


Fig. 2. Regression lines *a*, *b*, *c* and *d* (*cf.* text) corresponding to mutations from w^+ to w and to fractional w. Crosses mark observed mutation frequencies. At $x = 0$ the cross corresponds to series K and the dot to the observed frequency in the pooled series K + X + XI.

not normal, and normal distribution within classes is one of the prerequisites for the applicability of the ordinary method. On the other hand, it is necessary to assume that there exists a value which, at least approximately, may be used as an estimate of the average variance within *all* classes. At the very low probabilities with which one has to

reckon in the present case, however, the variances within doses change considerably from dose to dose. To overcome these difficulties we have proceeded according to two different methods.

In the first place, we have made χ^2 tests for the comparisons of the observed mutation rates and the rates expected according to the regression lines *a*. This method is quite permissible, since the total number of mutations at each dose is large enough to give the averages a normal distribution. Secondly, we have applied a special method

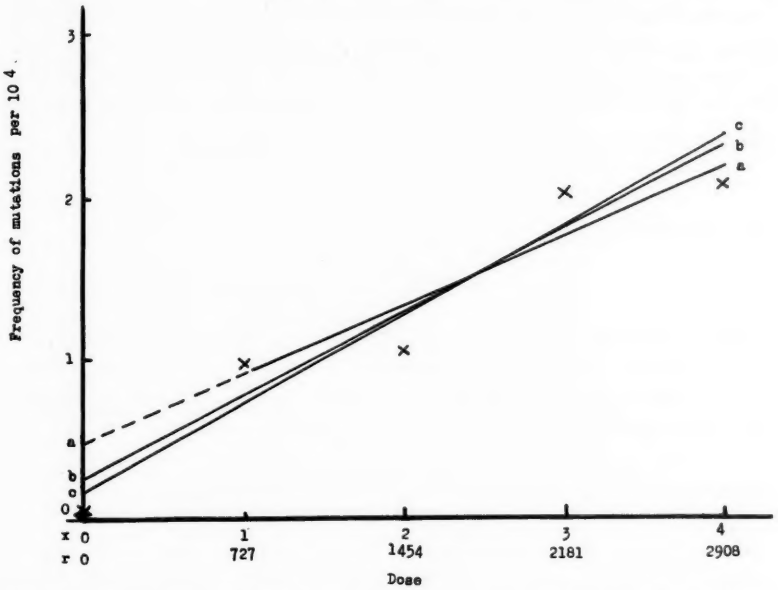


Fig. 3. Regression lines *a*, *b* and *c* (*cf.* text) corresponding to mutations from f^+ to f. Crosses mark observed mutation frequencies. At $x = 0$ the cross corresponds to series K and the dot to the observed frequency in the pooled series K + X + XI.

which we owe to Dr. H. CRAMÉR. This method is only an approximate one, but it is of a very great value in cases like the present one. His method is as follows.

Denoting by n the number of mutations at a certain dose and by N the total number of flies within this dose, the frequency of mutations y is $\frac{n}{N}$. Since this fraction $\frac{n}{N}$ is very small, a good estimate of the variance within the doses in question is given by the formula

$$s^2 = \frac{y}{N} = \frac{n}{N^2}$$

Furthermore, averages of x and y differing from the arithmetic means and a new regression coefficient are defined as follows

$$\bar{x}_C = \frac{S^x_{s^2}}{S^1_{s^2}} \qquad \bar{y}_C = \frac{S^y_{s^2}}{S^1_{s^2}} \qquad b_C = \frac{S(x - \bar{x}_C)(y - \bar{y}_C)}{S\frac{(x - \bar{x}_C)^2}{s^2}}$$

where the sums are to be extended over all doses. (In order to make the least possible errors of computation $\frac{1}{s^2}$ should be computed as $\frac{N^2}{n}$.) Finally, a new regression line is given by the equation

$$Y_C = \bar{y}_C + b_C(x - \bar{x}_C)$$

The expression

$$\chi^2_C = S\frac{(y - Y_C)^2}{s^2}$$

is then a measure of the deviation from linearity and it is distributed as an ordinary χ^2 with $k - 2$ degrees of freedom, where k is the number of classes (number of doses). To test whether the true regression line passes through a given point x_o, y_o we compute

$$t_C = \sqrt{k-2}\,\frac{\bar{y}_C + b_C(x_o - \bar{x}_C) - y_o}{\chi^2_C \sqrt{\frac{1}{S^1_{s^2}} + \frac{(x_o - \bar{x}_C)^2}{S\frac{(x - \bar{x}_C)^2}{s^2}}}}$$

where t_C is distributed as Student's t with $k - 2$ degrees of freedom.

Here it may be observed that, when dealing with mutation rates of orders of magnitude as those found in our series VII, V, III and I (Table 2), the difference between the ordinary regression line and the CRAMÉR regression line is very small, and we may accordingly substitute in the formula for ${\chi_C}^2$ and t_C the more easily calculable ordinary regression function Y for Y_C. In case the rate is very much smaller, for instance of the order of magnitude of our forked mutations in series K (Table 2), the discrepancy between Y and Y_C is, however, not small enough to be neglected. When, as in some cases, we have found

no mutations at all, *i. e.* when $y = 0$, the formulas are not applicable, since, at the dose in question, $\frac{1}{s^2}$ will then be infinitely large.

Dr. CRAMÉR has deduced another related formula, which is of value to have access to — although it has not been applied in our study. It may, for instance, be asked whether the true regression line passes through the point $x = 0$, $y = 0$. (Note, in our case we know that spontaneous mutations certainly do occur, and that hence the true regression line cannot pass through this point; but if the true regression line attains a value y for $x = 0$, which is very close to 0, the test below will still be applicable.) The CRAMÉR test then reduces to a computation of the regression coefficient

$$B = \frac{S\frac{xy}{s^2}}{S\frac{x^2}{s^2}}$$

and from this the expression

$$S\frac{(y - Bx)^2}{s^2}$$

which is distributed as χ^2 with $k - 1$ degrees of freedom.

TABLE 6. *Linearity test of regression lines a.*

Mutant	Equation of regression line	χ^2 test. 4 degrees of freedom		CRAMÉR test. 2 degrees of freedom	
		χ^2	P	χ^2_C	P
white	$Y = 10^{-4}(0{,}4219\,x + 0{,}5779)$	0,19	$> 0{,}99$	0,22	0,90
white + fractional white	$Y = 10^{-4}(0{,}4990\,x + 0{,}7417)$	0,02	$> 0{,}99$	0,03	0,99—0,98
forked	$Y = 10^{-4}(0{,}4284\,x + 0{,}4801)$	2,33	0,70—0,50	2,58	0,30—0,20

Table 6 shows the results of the linearity tests for the regression lines a, computed from the rates at the four doses $x = 1, 2, 3, 4$. For white and for white + fractional white we find an extremely good fit to linearity by using either method. (In fact, the high values of the P's found here as well as in some cases of Table 4 are noteworthy as

showing that such high values really do occur on random grounds.) In the case of forked the fit is also good. Here, however, the two tests give somewhat different results. Either the χ^2 test exaggerates the fit or the CRAMÉR test underestimates the agreement between observation and linearity. In any case, the agreements with linearity are so good that we may be sure that they would have held even if the material had been many times larger.

We may now proceed to the important question of how the observed rates at doses $x = 1, 2, 3, 4$ — or, more correctly, how the regression lines a — agree with the rates observed at $x = 0$. The rates which we have found in series K (Table 2) are the spontaneous rates, *i. e.*, in this case $x = 0$. But as the doses in series X and XI (Table 2) are very low — in x-units they are equal to 0,022 and 0,011, respectively — and as these series were started as a kind of extended control series, which, as seen from Table 2, were found to tally well with series K, we will also study the results in the pooled series K + X + XI for which we may also regard x as equal to 0. The present question may be tested, as the foregoing one, either by a χ^2 test or by a CRAMÉR test. These two tests are now, however, from one point of view of essentially different nature. The CRAMÉR t_C test, computed for $x = 0$, tells us the probability that the true regression line passes through a specified point on the ordinate axis, our observed frequency for $x = 0$. Thus it does not take into account the magnitude of observations at $x = 0$, *i. e.*, in our series K, X and XI. The χ^2 tests, on the other hand, consist, as before, of testing the disagreement between observations in these series and the frequencies expected at $x = 0$ according to the regression lines a. Hence these tests do take the magnitude of observations at

TABLE 7. CRAMÉR *test of the probability that the true regression lines corresponding to lines a (Table 6) pass through observed frequencies at dose* $x = 0$. *All degrees of freedom* = 2. *(As there were no whites observed in any of the series K, X, XI the tests for white are common for the two combinations of series K and* K + X + XI.)

Mutant	Series	t_C	P
white		6,96	0,02
white + fractional white	K	22,12	0,01 — 0,001
	K + X + XI	20,16	0,01 — 0,001
forked	K	1,40	0,30 — 0,20
	K + X + XI	1,52	0,30 — 0,20

TABLE 8. *χ^2 test for the comparison at dose $x = 0$ (series K, X, XI; Table 2) between observed frequencies and frequencies expected according to regression lines a (Table 6). All degrees of freedom = 1.*

Mutant	Series	χ^2	P
white	K	8,77	0,01 — 0,001
	K + X + XI	17,40	< 0,001
white + fractional white	K	11,39	< 0,001
	K + X + XI	18,79	< 0,001
forked	K	5,51	0,02
	K + X + XI	12,70	< 0,001

$x = 0$ into account. It is therefore to be expected that even in cases when the lines a fit the observed frequencies according to the CRAMÉR t_C test, the χ^2 test may show a significant discrepancy.

The results of these tests are shown in Tables 7 and 8. The CRAMÉR t_C tests show a distinct disagreement between lines a and observation at dose $x = 0$ in the case of white and, a still more distinct one, in the case of white + fractional white. In the case of forked, however, there is good agreement. The χ^2 tests, Table 8, on the other hand, show distinct disagreements in all three cases.

We have also made a combined test which will serve to strengthen the validity of our conclusions. First we have computed the y_o value for $x_o = 0$ which makes $t_C = 0{,}05$, and then we have made a χ^2 test for the comparison between observed frequencies at $x = 0$ and the frequency corresponding to y_o. To make this test is, with regard to the P values in Table 7, of interest only in the cases of white and white + fractional white. The results of the tests are shown in Table 9. The

TABLE 9. *χ^2 test for the comparison at dose $x = 0$ (series K, X, XI; Table 2) between observed frequencies and frequencies expected at the 0,05 points below regression lines a. All degrees of freedom = 1.*

Mutant	Series	χ^2	P
white	K	3,39	0,10 — 0,05
	K + X + XI	6,72	0,01 — 0,001
white + fractional white	K	9,17	0,01 — 0,001
	K + X + XI	14,43	< 0,001

disagreements are still very noticeable: the observed frequencies are thus significantly smaller than the »expected» values, which themselves are significantly smaller than the values expected according to lines *a*.

The conclusion to be drawn from the present analysis is then that, although there is a very good agreement between observation and the linearity hypothesis at the doses $x = 1, 2, 3, 4$ ($r = 727, 1454, 2181, 2908$), the observations in the control series ($r = 0, 16, 8$) do not fit the same line.

It is one of the necessary principles of statistics that the partition of a material into groups which are to be compared should not be made on the basis of the observed results themselves, but only on *a priori* grounds. It must, for instance, be strictly forbidden to compare the parts which are most extreme in either direction only because they are the most extreme ones. Now, it is, in itself, biologically sound to contrast, in experiments of any kind, controls *versus* experimental results. Hence we should have been quite entitled to make the comparisons just made even if we had planned the control series from the outset and started them simultaneously with the X-ray series. We must add to this the fact, however, that we started these controls *because* we found the regressions from our irradiation experiments to correspond to mutation frequencies at $x = 0$, which seemed us to be higher than could be expected as spontaneous frequencies. We should thus have been quite justified in contenting ourselves with the analyses made. In spite of this, however, it is of interest to investigate the linearity of the regressions based on the mutation rates at the five doses $x = 0, 1, 2, 3, 4$. For each of the three mutational combinations studied we have therefore computed 2 more regression lines based on the rates at $x = 0$ as well as on those at the doses $x = 1, 2, 3, 4$. They are the lines *b* for which series K has been used for $x = 0$, and the lines *c* for which the pooled series K + X + XI have been used for $x = 0$ (Figs. 1—3).

The χ^2 tests of the regression lines *b* and *c* are found in Table 10. The *P* values are, as expected, now larger than those obtained in the comparisons for lines *a* (Table 8), but in the case of white and also in that of white + fractional white they are still rather small. It may also be remarked that the largest part of the χ^2s comes from the comparisons at $x = 0$. Even these lines, *b* and *c*, do not agree very well with the observed control frequencies.

Still one more comparison has been made. The CRAMÉR χ_C^2 test is applicable to $x = 0$ when y is not 0. This is the case for white + fractional white when we are using the pooled series K + X + XI, in

TABLE 10. *Linearity test of regression lines b and c. All degrees of freedom = 5.*

Mutant	Regression line	Equation of regression line	χ^2	P
white	*b*	$Y = 10^{-4}\ (0{,}5288\,x + 0{,}5224)$	9,67	0,10 — 0,05
	c	$Y = 10^{-4}\ (0{,}5679\,x + 0{,}2222)$	9,52	0,10 — 0,05
white + fractional white	*b*	$Y = 10^{-4}\ (0{,}6315\,x + 0{,}4142)$	8,05	0,20 — 0,10
	c	$Y = 10^{-4}\ (0{,}6661\,x + 0{,}3287)$	9,25	0,10
forked	*b*	$Y = 10^{-4}\ (0{,}5088\,x + 0{,}2878)$	6,04	0,30
	c	$Y = 10^{-4}\ (0{,}5465\,x + 0{,}1977)$	8,64	0,20 — 0,10

TABLE 11. CRAMÉR *linearity test in the case of white + fractional white for the doses $x = 0$, 1, 2, 3, 4, using the combined series K + X + XI (Table 2) for $x = 0$ (Fig. 2, line d). 3 degrees of freedom.*

Equation of regression line	χ^2_C	P
$Y_C = 10^{-4}\ (0{,}8703\,x + 0{,}0714)$	6,69	0,10 — 0,05

which 2 mutants were found (Table 2). We have computed the CRAMÉR line Y_C for this case (line *d*, Fig. 2). As a result of the very heavy weight which this test gives to the extremely low observed mutation frequency at $x = 0$, this line passes very close to the observed control value and rather far above the observed points for the higher doses. The result of the test is seen in Table 11, showing a rather bad fit.

If we had based our analyses solely on the lines *b*, *c* and *d* we should have concluded that the fits were bad, though not disproving the linearities. Together with the analyses based on lines *a* it seems to us to be very difficult to escape from interpreting our data as showing that the linearity between mutation frequency and dose, being very good when the control data are not included, does not hold good when these controls are included in the considerations.

SUMMARY.

(1) Wild males of *Drosophila melanogaster* have been X-rayed at the doses 727 *r*, 1454 *r*, 2181 *r* and 2908 *r* and mated to females homo-

zygous for white and forked or to females homozygous for yellow, white, forked and Beadex. The female progeny, which (including gynandromorphs) consisted of a total of 1,216,698 individuals, has formed the basis of a statistical investigation of mutation frequencies in the two single loci w^+ and f^+.

(2) A method of registration which has enabled the study of variation within doses is described.

(3) It is shown that this variation is strictly Poisson, and that the variation between different instances of irradiation has been cancelled by repeating each dose a number of times.

(4) For the three mutational combinations $w^+ \rightarrow w$, $w^+ \rightarrow w$ or fractional w and $f^+ \rightarrow f$, an analysis has been made of the relation between dose and mutation frequency. It is found to be strictly linear.

(5) When the results are included of control experiments — which here means frequencies of spontaneous mutations and of mutations at the low doses $r = 8$ and $r = 16$ — it is very difficult to escape from the conclusion that this linearity no longer holds.

(6) In a second part of the present study, soon to be published, a number of special cases from the present investigation will be presented, and their evaluation with regard to the mode of mutational processes will be discussed. The results of the present part of the investigation will be included in that discussion.

LITERATURE CITED.

1. CASPARI, E. and STERN, C. 1948. The influence of chronic irradiation with gamma-rays at low dosages on the mutation rate in *Drosophila melanogaster*. — Genetics 33, 75—95.
2. FISHER, R. A. 1936. Statistical methods for research workers. 6th ed. 339 pp. — Edinburgh.
3. HANSON, F. B. and HEYS, F. 1929. An analysis of the effects of the different rays of radium in *Drosophila*. — Amer. Nat. 63, 201—213.
4. — and — 1932. Radium and lethal mutations in *Drosophila*. Further evidence of the proportionality rule from a study of the effects of equivalent doses differently applied. — Amer. Nat. 66, 335—345.
5. LEA, D. E. 1946. Actions of radiations on living cells. 402 pp. — Cambridge.
6. MULLER, H. J. 1940. An analysis of the process of structural change in chromosomes of *Drosophila*. — Journ. of Gen. 40, 1—66.
7. — 1941. Induced mutations in *Drosophila*. — Cold Spring Harbor Symp. Quant. Biol. 9, 151—165.

8. OLIVER, C. P. 1930. The effect of varying the duration of X-ray treatment upon the frequency of mutations. — Science 71, 44—46.
9. — 1932. An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. — Zschr. f. ind. Abst.- u. Vererb.-lehre 61, 447—488.
10. RAYCHAUDHURI, S. P. 1939. The validity of the BUNSEN-ROSCOE law in the production of mutations by radiation of extremely low intensity. — Proc. 7th Int. Cong. Gen. Edinburgh 246.
11. — 1944. The validity of the BUNSEN-ROSCOE law in the production of mutations by radiation of extremely low intensity. — Proc. Royal Soc. Edinburgh 62, 66—72.
12. SPENCER, W. P. and STERN, C. 1948. Experiments to test the validity of the linear *r* dose/mutation frequency relation in *Drosophila* at low dosage. — Genetics 33, 43—74.
13. STEVENS, W. L. 1942. Accuracy of mutation rates. — Journ. of Gen. 43, 301—308.
14. TIMOFÉEFF-RESSOVSKY, N. W. 1931. Einige Versuche an *Drosophila melanogaster* über die Art der Wirkung der Röntgenstrahlen auf den Mutationsprozess. — Roux' Arch. Entw.-Mech. 124, 654—665.
15. — 1934. Einige Versuche an *Drosophila melanogaster* über die Beziehungen zwischen Dosis und Art der Röntgenbestrahlung und der dadurch ausgelösten Mutationsrate. — Strahlen-Therapie 49, 463—478.
16. — 1937. Experimentelle Mutationsforschung in der Vererbungslehre. 181 pp. — Dresden und Leipzig.

CONTENTS.

I. Introduction 163
II. Material and methods 165
III. The classification of the progeny of X-rayed males, and sources of error in this classification 167
IV. The registration of the results and their statistical evaluation 171
V. The linear dose-mutation frequency relation 177
Summary 187
Literature cited 188

SYNTHESIS OF PHLEUM PRATENSE L. FROM P. NODOSUM L.

BY *HEDDA NORDENSKIÖLD*

INSTITUTE OF PLANT SYSTEMATICS AND GENETICS, ROYAL AGRICULTURAL COLLEGE OF SWEDEN, UPSALA

PREVIOUS investigation of the subgroup *Euphleum* of the genus *Phleum* (NORDENSKIÖLD, 1945) gave rise to the assumption that the hexaploid *P. pratense* must have arisen from the diploid *P. nodosum* by repeated doubling of its chromosomes. To obtain further proof of that theory, efforts were made for several years to produce a hexaploid *P. nodosum* from the diploid species. By the aid of repeated colchicine treatments of the diploid *P. nodosum*, followed by crosses between the polyploids obtained, I succeeded, in the summer of 1947, in getting two hexaploid plants.

Origin of the hexaploid P. nodosum. — In order to get this hexaploid, a tetraploid strain of *P. nodosum* was first produced by the aid of colchicine treatment. The material used for this purpose was a strain of »Svalöf's pasture timothy». The colchicine treatment was carried out in the summer of 1940, partly on seeds, and partly on young seedlings. The concentrations of colchicine used lay between 0,1 and 0,025 per cent, and the duration of the treatment varied between 1 and 6 days. Progenies were taken from the treated plants in the summers of 1941 and 1943, and both these progenies contained tetraploid as well as diploid plants. The first strain of tetraploid *P. nodosum*, obtained in 1941, was acquired from the cross between the male-sterile *P. nodosum* No. 584, a plant with a high percentage of unreduced gametes caused by asyndesis (NORDENSKIÖLD, 1945, p. 9), as the female parent, and two colchicine-treated plants as the male parents. This tetraploid strain, containing 14 seedlings, thus originated from only three plants, one of which was the male-sterile plant No. 584. As *P. nodosum* is a cross-breeder, it was desirable to have more than just these three parent plants in the strain. In order to get a progeny from some other plants treated, seeds were taken from such plants, crossed together in a cage in the summer of 1943. About a hundred tetraploid seedlings were obtained from these crosses, and two of the plants treated, which had not earlier given any tetraploid progeny, now gave such. This new

strain of tetraploids was crossed together with the old one of 1941 so as to get a new tetraploid strain of *P. nodosum* with a greater variation.

For the purpose of getting a hexaploid *P. nodosum* from these tetraploid strains several different methods were tried.

The first method used was to cross the male-sterile plant of *P. nodosum* No. 584 with the tetraploid strains. This plant, No. 584, not only formed unreduced gametes with 14 chromosomes but also gametes with 28 chromosomes, the double somatic chromosome number of the plant (NORDENSKIÖLD, 1945, p. 9). If these latter gametes should function with pollen from a tetraploid male plant, a hexaploid plant would be obtained. Even at the production of the first strain of tetraploid *P. nodosum*, the cross with plant No. 584 was made with the intention of getting hexaploid plants directly from that cross. But no hexaploids were obtained by this procedure.

The second method tried was to examine twin plants from the tetraploid strain of *P. nodosum*. In many species one of the two twin plants sometimes has a deviating chromosome number, and then it is usually triploid, as compared with the mother plant. In *P. pratense*, too, triploid twin plants have been found (MÜNTZING, 1938). To obtain such a triploid from the tetraploid strain of *P. nodosum* about 100000 seedlings were examined and 128 pairs of twins obtained. But none of these twin plants was triploid as compared with the mother plant. Only five twin plants with a deviating chromosome number were found. All these were, however, haploid as compared with the mother plant, *i. e.* had 14 chromosomes.

The last method used to obtain a hexaploid *P. nodosum* was to treat tetraploid seedlings with colchicine. This treatment was carried out twice, during the summers of 1942 and 1945. In the spring of 1942 the first strain of tetraploid *P. nodosum* of 1941 was used. No result was obtained. In the spring of 1945 the colchicine treatment was repeated, and the new conjoined strain of *P. nodosum* of 1944 was used. This time a positive result was obtained. One of the plants treated had two ears, which, judging from the size of pollen and anthers, must have been at least partly chromosome-doubled. In the progeny of these ears two plants were hexaploid. These two plants must have been produced in the chromosome-doubled part of the ears, fertilized by pollen from tetraploid flowers. The plants treated had been crossed with tetraploid *P. nodosum* in order to get this cross. The two hexaploid plants exhibited the same habitus mutually, as well as compared with the tetraploid strain of *P. nodosum* from which they originated. This is

Fig. 1. To the left the two plants of hexaploid *P. nodosum*, to the right a wild type of *P. pratense*.

pointed out to display the fact that the two hexaploid plants must have originated from the tetraploid strain of *P. nodosum*.

Morphological appearance of the hexaploid P. nodosum. — The hexaploid *P. nodosum* is habitually very similar to the low, slender wild type of *P. pratense* (Fig. 1). The ears are thicker than the usually very thin ears of *P. nodosum*, thus resembling the ears of the wild *P. pratense* (Fig. 2). Comparative measurements have revealed that the absolute length of the awns is the character showing the best and only significant distinction between *P. nodosum* and *P. pratense*, though some transitional forms have been found (NORDENSKIÖLD, 1945, pp. 95—98). The hexaploid *P. nodosum* has clearly longer awns than the diploid, and its awn-length is comparable with some types of *P. pratense* (Table 1, Fig. 3). On an average the awn-length of the tetraploid *P. nodosum* lies

Fig. 2. Upper row: Ears of the two plants of hexaploid *P. nodosum*. Lower row: The first group of two ears are of diploid *P. nodosum*, the second group of three ears are of a wild type of *P. pratense*. — About $^3/_5$ natural size. — Photo: E. HESSELMAN.

between that of the diploid and of the hexaploid (Table 1). The length of the awns as well as the length of the glumes was determined here by measuring these two characters from 100 spikelets of each plant investigated. The length of the glumes did not show any marked difference between *P. pratense* and *P. nodosum* (NORDENSKIÖLD, 1945, pp. 95—98); so it could not be used as a distinctive character separating the two

TABLE 1. *Length of the awns in P. pratense and in different polyploids of P. nodosum.*

Strain \ Length of awns in mm.	0,6	0,7	0,8	0,9	1,0	1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8
P. pratense, cult.			1			2	1	2	2	1		1	1
P. » wild				2	4	1	1	1	2				
P. nodosum, hexaploid ...									1	1			
P. » tetraploid ...	1	1	3	1			2						
P. » diploid	4	1	1	2		2	1						

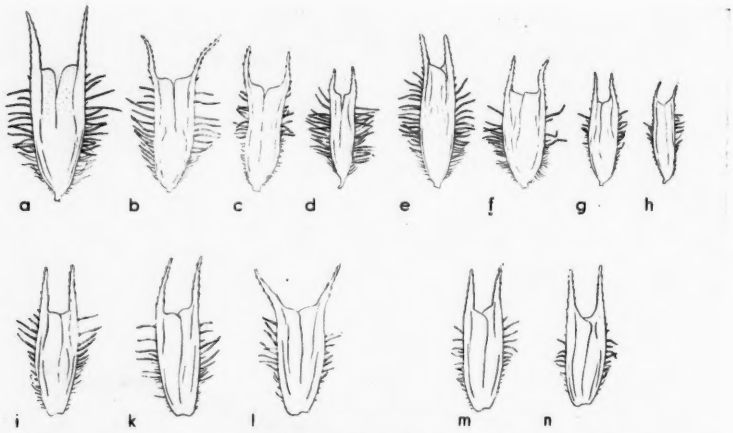


Fig. 3. Spikelets of *P. pratense*, diploid, and hexaploid *P. nodosum*: *a—d P. pratense*, *e—h* diploid *P. nodosum*, *i—n* hexaploid *P. nodosum*. — Magnification: Five times natural size.

species. Yet taxonomists have very often used it as such a character, and that is the reason why comparative measurements of this kind have been made (Table 2). The glumes of the hexaploid *P. nodosum* have about the same length as the larger ones of the wild type of *P. pratense* as well as those of *P. nodosum*. In the two hexaploid plants the length of the awns in relation to that of the glumes is 44 and 47 per cent respectively, *i. e.* higher than in any diploid *P. nodosum* hitherto found. On the other hand, these figures are of the same order of magnitude as those of *P. pratense* (NORDENSKIÖLD, 1945, p. 98). Other measurements, such as the size of the ears and leaves or height of the plants, were not made on account of the great modificatory variability of these organs. As there were only two plants of the hexaploid *P. nodosum*,

TABLE 2. *Length of the glumes in P. pratense and in different polyploids of P. nodosum.*

Strain \ Length of glume in mm.	2,6	2,7	2,8	2,9	3,0	3,1	3,2	3,3	3,4	3,5	3,6
P. pratense, cult.....						2	1		2	6	
P. » , wild	3		3		2	1	1	1			
P. nodosum, hexaploid					1				1		
P. » , tetraploid.....					1	2	5				
P. » , diploid		1	1		2	3	2	2			

and as these plants had not yet been divided up into clones, the errors incidental to comparative measurements of that kind would have been too great.

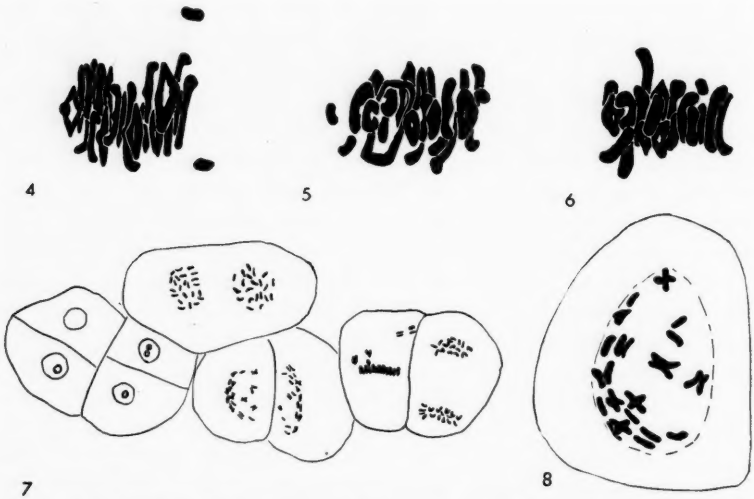
Fertility of the hexaploid P. nodosum and its capability of being crossed. — The investigations into the pollen fertility of the two hexaploid plants of *P. nodosum* gave 87 and 97 per cent of good pollen, respectively. That means somewhat reduced pollen fertility for one of the plants, the other having just as good pollen as normal *P. pratense*. This species generally has about 95 per cent of good pollen. The reduced pollen fertility may depend on the extra chromosomes (see below) which both the hexaploids contain. However, polyploids recently induced by artificial means often have a reduced fertility, independently of extra chromosomes.

The female fertility of the hexaploid *P. nodosum* was, in spite of the extra chromosomes, quite good. Crosses between the two hexaploids gave 5—10 seeds per cm. of ear. When the plants were crossed with *P. pratense* the seed-setting was higher and yielded 15—20 seeds per cm. of ear. That this cross with *P. pratense* gave a higher fertility than the cross between the two hexaploid plants of *P. nodosum* must depend on the fact that the hexaploids were siblings. The seed-setting of the cross between these siblings will be reduced on account of *P. nodosum* being self-sterile. This species as well as its tetraploid strain is highly self-sterile and there is no reason to think the state to be different in hexaploid *P. nodosum*. This self-sterility does not affect the cross with the unrelated *P. pratense*.

The seed-setting of *P. pratense* is very variable. While the cultivated strains usually have a seed-setting of 30—40 or more seeds per cm. of ear, the wild strains of *P. pratense* are very often more or less sterile. A seed-setting of less than 10 seeds per cm. of ear is common among these types.

Crosses between the hexaploid *P. nodosum* and the diploid *P. nodosum* or any other species of *Phleum* have not yet been carried out.

Cytological investigation of the hexaploid P. nodosum. — After a careful investigation of the somatic chromosomes of the root-tips, the two hexaploid plants of *P. nodosum* proved to have 44 and 45 chromosomes, respectively. These extra chromosomes of the hexaploid may have been transferred to the plant by both the parents. As the father plants, however, were unknown, only the mother plant could be investigated. The chromosome number of the mother plant was accordingly investigated and proved to be 27 instead of 28. Plants deviating



Figs. 4—8. Meiosis of the hexaploid *P. nodosum*. — Fig. 4. First metaphase of the hexaploid *P. nodosum* with 44 chromosomes forming 21 bivalents and 2 univalents. — Fig. 5. First metaphase of the hexaploid *P. nodosum* with 44 chromosomes showing, besides bivalents, one quadrivalent and two univalents. — Fig. 6. First metaphase of the hexaploid *P. nodosum* with 45 chromosomes showing bivalents and one univalent. — Fig. 7. Later phases of meiosis of the hexaploid *P. nodosum* with 45 chromosomes. In the same view is seen a tetrad, a second metaphase, a dyad with disturbances and reversion of the second division, and another dyad with a normal second anaphase to the right and a retarded second metaphase to the left. — Fig. 8. One of the cells with reversion at the second meiotic division enlarged. — Magnifications: Figs. 4—6, 8. × 2400; Fig. 7. × 1000.

in chromosomes are rather common in the strain of tetraploid *P. nodosum*, and the colchicine treatment was made on uninvestigated seedlings. The aberrant chromosome number of the mother plant, however, must have caused this plant to form gametes with varying chromosome numbers, in the normal as well as in the chromosome-doubled sections of the ears.

Meiosis of the two hexaploid plants was surprisingly regular, especially the first metaphase. In the plant with 44 chromosomes, the two extra chromosomes were usually observed as two univalents. The rest of the chromosomes mostly formed rod- and ring-shaped bivalents (Figs. 4 and 5). In some cells a quadrivalent was formed instead of two bivalents (Fig. 5). Of 70 cells investigated, 15 contained a quadrivalent, but in no cases were two quadrivalents with certainty observed in one cell. Later stages of meiosis were mostly regular, except for some of the

univalents lagging between the poles at both the first and the second anaphase. The tetrad-formation also took place in an ordinary way in this plant.

The plant with 45 chromosomes showed the same almost regular first metaphase. Usually all three univalents were seen, but in many cases only one (Fig. 6). The rest of the chromosome formed bivalents; only in a few cells was a quadrivalent seen. In this plant the second division was not so regular as in the one first investigated. The most striking anomaly was the fact that the rhythm of division was lost at the later stages. In the same anther cells in all stages from second metaphase to tetrads were formed (Fig. 7). Besides, dyads were very often formed instead of tetrads. They arise through disturbances and reversions of the second division. It seems as though the interkinesis, or sometimes the metaphase, is turned into an extended prophase-like stage. The chromosomes already split are shortened, and the chromosome-halves usually lie near each other, parallel or forming a cross (Fig. 8). The same phenomenon has earlier been observed in the meiosis of triploid *P. nodosum* (NORDENSKIÖLD, 1941, p. 24). This plant with the more irregular meiosis was the one of the hexaploids which had a reduced pollen-fertility.

Discussion. — In my earlier work on the subgroup *Euphleum* within the genus *Phleum* (NORDENSKIÖLD, 1945) I called attention to the fact that the morphological differences between the two species *P. pratense* and *P. nodosum* are of a quantitative character, and that no certain qualitative differences have been found. These quantitative differences present themselves most obviously in the size of stem, leaves and ears, the vigour of the plant, the length of the awns, *etc.*, *P. pratense* being the more robust of the two species. Diversities of this kind naturally call to mind the differences usually characterizing various experimentally produced polyploids as compared with their normal diploids. Since *P. pratense* is the more robust of the two species, it thus differs from *P. nodosum* in the same way as artificially induced polyploids do from the original diploids.

On studying the hybrids between *P. nodosum* and *P. pratense* it was found that, when their chromosome numbers were high, they were morphologically very similar to *P. pratense*, independent of the origin of the chromosomes. Thus the hybrids between *P. nodosum* and *P. pratense*, with 35 and 49 chromosomes respectively, and the hybrid between triploid *P. nodosum* and *P. pratense*, with 42 chromosomes, were mutually very similar, and were impossible to distinguish from

real *P. pratense* (NORDENSKIÖLD, 1945, pp. 12 and 26). This was the case although only 21 of the chromosomes originated from *P. pratense*, and the rest (14, 28, and 21 respectively) from *P. nodosum*. The 28-chromosomal hybrid between *P. pratense* and *P. nodosum*, on the contrary, was morphologically of an intermediate appearance, in spite of the fact that 21 of the 28 chromosomes originated from *P. pratense* (NORDENSKIÖLD, 1945, p. 22). In the corresponding crosses between the diploid *P. alpinum* L., GAUD. and *P. pratense*, which gave hybrids with 35 and 49 chromosomes respectively, the hybrid having 35 chromosomes was morphologically intermediate between the parents, and the one with 49 chromosomes was mostly like a coarse, polyploid *P. alpinum*. This last-mentioned hybrid has 28 of its 49 chromosomes from *P. alpinum*. The hybrids between *P. pratense* and the tetraploid *P. commutatum* GAUD., with 35 and 49 chromosomes respectively, were of the same type. The one with 35 chromosomes was morphologically intermediate between the parents, the one having 49 chromosomes was most like *P. commutatum* (NORDENSKIÖLD, 1945, pp. 34 and 36). Moreover, both the hybrids of this combination were almost sterile, a character which none of the other high-chromosomal hybrids ever had. These differences in morphological appearance of the hybrids between *P. pratense* and *P. nodosum*, on the one hand, and *P. alpinum* and *P. commutatum*, on the other, are most easily explained by assuming all three genomes of *P. pratense* to be similar to those of *P. nodosum* but dissimilar to those of *P. alpinum* and *P. commutatum*.

In meiosis of the hybrids, the pairing of chromosomes between *P. pratense* and *P. nodosum* also indicate that the three genomes from *P. pratense* must be mutually similar and like those of *P. nodosum*. The hybrid between these species with 28 chromosomes proved to have a complete autosyndesis with usually 14 bivalents, and the tetraploid cross-product between this hybrid and tetraploid *P. nodosum* had a meiosis of the very same configurations (NORDENSKIÖLD 1945, pp. 64 and 65). The meiosis of the high-chromosomal hybrids between *P. nodosum* and *P. pratense* also had a pairing of chromosomes, which is most easily explained by the assumption that all the chromosome sets of *P. pratense* are able to pair mutually as well as with those of *P. nodosum*.

Morphologically the appearance of the experimentally produced hexaploid *P. nodosum* confirms the assumption that *P. pratense* must have arisen from *P. nodosum* by chromosome-doubling. The two hexaploids look like a very robust and coarse plant of *P. nodosum*, and as

such they are almost impossible to distinguish from certain types of wild *P. pratense* (Figs. 1, 2 and 3). The length of the awns, the best characteristic by which to discriminate *P. pratense* from *P. nodosum*, is much longer in the hexaploid than in the diploid *P. nodosum*, and this awn-length of the hexaploids is directly comparable with that of *P. pratense*.

The meiosis and especially the first metaphase of the hexaploid *P. nodosum* is surprisingly regular. Except for the univalents derived from the extra chromosomes, mainly bivalents are formed and only in a few cells multivalents — and, in such cases, exclusively quadrivalents. These facts indicate that meiosis will probably become regular in later generations when the chromosome number of the hexaploids has been stabilized.

Taking into consideration all the facts given in detail above, the conclusion will be that at least the wild type of *P. pratense* must have arisen from *P. nodosum* solely by means of chromosome doubling. Then the hexaploid *P. nodosum* must be considered a synthesized *P. pratense*. That this synthesized *P. pratense* is more like the wild type of *P. pratense* than the cultivated one, only supports the theory.

In the previous literature (GREGOR and SANSOME, 1930), a 42-chromosomal plant originating from an F_1 hybrid between *P. nodosum* and *P. commutatum* was suggested to be a synthesized *P. pratense*. This plant was also supposed to be an amphidiploid between *P. commutatum* and *P. nodosum*, though, most probably, it must be looked upon as a cross between the F_1 hybrid and *P. pratense* (NORDENSKIÖLD, 1945, p. 125; GREGOR, 1945/46). As a cross product of *P. pratense* it was morphologically much more like this latter species than the real amphidiploid between *P. commutatum* and *P. nodosum*, which amphidiploid is not at all morphologically like *P. pratense* (Figs. 1 and 9) and consequently cannot be considered as this species synthesized.

Assuming *P. pratense* to have arisen as a hexaploid *P. nodosum* in nature, it is easy to point out several different paths along which a development of this kind might have taken place. The rather common occurrence of unreduced gametes in *P. nodosum*, and sometimes of such as have the double somatic chromosome number, indicates that polyploids must be relatively easily formed in this species. Triploid *P. nodosum* has arisen in a natural way in the experimental field by the aid of unreduced female gametes. Such a triploid plant can very easily turn into a hexaploid by means of chromosome doubling. Another way in which a hexaploid plant might directly arise in nature from

Fig. 9. To the left the amphidiploid between *P. nodosum* and *P. commutatum*, to the right the hexaploid *P. nodosum*.

P. nodosum is by the fusion of a gamete with the double somatic chromosome number (28) and an unreduced one (14). Such a cross combination never appeared in the experimental field, but this fact does not prove that it is impossible for it to arise.

The plants with a tendency to form unreduced gametes have often had a reduced ability of forming bivalents in meiosis. The univalents then formed disturb the meiosis, causing the formation of unreduced gametes. Formation of univalents seems to be caused by a reduced ability of chiasma formation (NORDENSKIÖLD, 1945, p. 53). It is possible that this reduced ability of chiasma formation, which seems to have a connection with the formation of unreduced gametes in diploids, might in polyploids cause a reduction of multivalent formation. This property

will promote the viability of the polyploids. The very low multivalent frequency in the meiosis of the hexaploid *P. nodosum* might depend on factors of this kind. They may also give an explanation of the lack of multivalents in meiosis of the hexaploid *P. pratense* (NORDENSKIÖLD, 1945, p. 57), as well as in the one with 63 chromosomes (MÜNTZING and PRAKKEN, 1940).

SUMMARY.

(1) Two plants of hexaploid *P. nodosum* have been produced by the aid of repeated colchicine treatments of seeds and young seedlings, followed by crosses between the polyploids obtained.

(2) The hexaploid *P. nodosum* has been compared with different strains of *P. pratense*. Morphologically it is very similar to certain types of wild *P. pratense*, both in its way of growth and vigour, and in the appearance of its ears and glumes. The awns are longer than those of the diploid *P. nodosum* and correspond in size to those of *P. pratense*.

(3) The fertility of the hexaploid *P. nodosum* is quite good, both male and female. The cross with *P. pratense* gave a good seed-setting.

(4) In the cytological investigation of the somatic chromosome number the two hexaploid plants of *P. nodosum* proved to have 44 and 45 chromosomes, respectively. In consequence of this the extra chromosomes appeared as univalents during meiosis. The other chromosomes mainly formed bivalents. Only in about 20 per cent of the cells was a quadrivalent found.

(5) In the discussion, attention is called to the following facts: (a) The morphological differences between *P. pratense* and *P. nodosum* are characterized throughout by quantitative characters, and no safe distinctive qualitative characters have been found. (b) The hybrids between *P. nodosum* and *P. pratense*, having 35, 42, and 49 chromosomes, are mutually very similar and are impossible to separate from real *P. pratense*. (c) The pairing of the chromosomes in the meiosis of the hybrids demonstrates that all three of the chromosome sets of *P. pratense* are able to pair reciprocally as well as with those of *P. nodosum*. (d) The hexaploid *P. nodosum* is morphologically very similar to some types of wild *P. pratense*. It has good fertility, almost regular meiosis, and is very easily crossed with *P. pratense*. On account of all these facts, it is proposed that the hexaploid *P. nodosum* must be considered a synthesized *P. pratense*.

(6) A certain connection between the formation of unreduced

gametes, depending on disturbed meiosis caused by a reduced chiasma formation, and the presence of polyploids in the genus *Phleum* are discussed. Different ways in which the hexaploid *P. pratense* might have arisen from *P. nodosum* in nature are also considered.

LITERATURE CITED.

1. GREGOR, J. W. 1945/1946. *Phleum alpinum* L. emend. GAUD. and *P. commutatum* GAUD. — Trans. and Proc. Bot. Soc. Edinburgh, Vol. 34, pp. 368—370.
2. GREGOR, J. W. and SANSOME, F. W. 1930. Experiments on the genetics of wild populations. II. *Phleum pratense* L. and the hybrid *P. pratense* L. × *P. alpinum* L. — Journ. of Genetics, Vol. 22, pp. 373—387.
3. MÜNTZING, A. 1938. Note on heteroploid twin plants from eleven genera. — Hereditas, Vol. XXIV, pp. 487—491.
4. MÜNTZING, A. and PRAKKEN, R. 1940. The mode of chromosome pairing in *Phleum* twins with 63 chromosomes and its cytogenetic consequences. — Hereditas, Vol. XXVI, pp. 463—501.
5. NORDENSKIÖLD, H. 1941. Cytological studies in triploid *Phleum*. — Bot. Notiser 1941, pp. 12—32.
6. — 1945. Cyto-genetic studies in the genus *Phleum*. — Acta Agriculturae Suecana, Vol. 1, pp. 1—138.

CONTENTS.

Origin of the hexaploid *P. nodosum* 190
Morphological appearance of the hexaploid *P. nodosum* 192
Fertility of the hexaploid *P. nodosum* and its capability of being crossed 195
Cytological investigation of the hexaploid *P. nodosum* 195
Discussion 197
Summary 201
Literature cited 202

CONTRIBUTIONS TO THE GENETICS OF THE Gp-CHROMOSOME OF PISUM

BY *ROBERT LAMM*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

INTRODUCTION.

IN the summer of 1941 an aberrant plant of *Pisum sativum* was found at the Horticultural Research Station at Alnarp, Sweden. From the hereditary analysis it appeared that the aberration was due to a new recessive gene, which I have given the symbol *cri* (*crispus*). This new gene belonged to the chromosome which has been denoted by LAMPRECHT (1948 b) as chromosome V or the *Gp*-chromosome. A further contribution to the genetics of this chromosome has been obtained from an F_2 progeny concerning the Extra Rapid translocation. The data about this F_2 have kindly been put at my disposal by Mr. E. NILSSON of the Seed Company J. E. Ohlsens Enke AB, Malmö, and these data have made it possible for me to determine the approximate position of the point of translocation (*Tr*) on the linkage map of the *Gp*-chromosome.

THE GENE Cri.

Origin and description. — The new mutation *cri* was found in the variety Juvel LD:s/41. This variety has been selected by the Seed Company AB L. Daehnfeldts Fröhandel, Hälsingborg, Sweden, from a cross between Delikatess and Witham Wonder. Both varieties have the length factors *le* Cy_1 $cy^c{}_2$, but the latter variety is slightly taller.

Plants homozygous for *cri* are, as is shown by Figs. 1—3, characterized by their crisp or folded leaves and stipula and their elongated leaflets of a rather obovate shape. The leaflets are more elongated than in the mutation *obovatus* described by LAMPRECHT (1945 b). Fig. 2 shows a single leaflet in slight magnification, and in Fig. 3 profiles of successive sections cut perpendicularly to the foldings of a leaflet are shown in a somewhat stronger magnification. The crisp structure also occurred on the inside of the pods, giving the covering membrane a tendency to crack at maturity. Although the pollen fertility of the *cri*-plants was quite normal, the seed setting was somewhat reduced, pro-

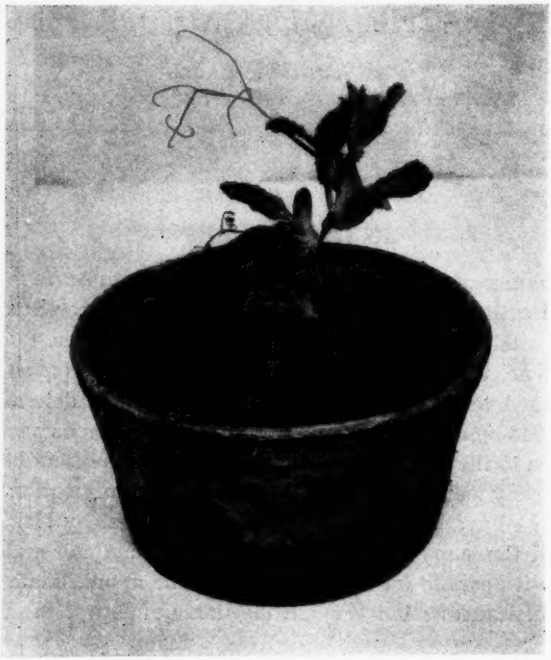


Fig. 1. Juvenile plant of line No. 21 homozygous for *cri*.

bably due to the folding of the carpels. Plants homozygous for *cri* are easily recognized even in the juvenile stage (see Fig. 1) and *cri* is thus a good marker gene. The pure line raised from the selfed seed of the *cri*-plant found in 1941 has been registered by me as line No. 21.

Linkage relations. — In order to localize the gene *Cri* crosses have been performed between line No. 21 (*le cri Gp Wa*) and lines No. 44 (*Le Cri gp Wa*) and No. 6 (*le Cri Gp wa*). A few other crosses have also been made, but these gave rise to comparatively small F_2 progenies, which, however, indicated free recombination between *Cri* and the genes *A*, *Am*, Cy_1 and *Wlo*. In the summer of 1944 I made the cross between lines No. 44 and No. 21. Two pods 44/65 and 44/66 were obtained, each containing two seeds. The progenies given in Table 1 originated from these pods.

The original F_2 data of Table 1 A show good single factor segreg-

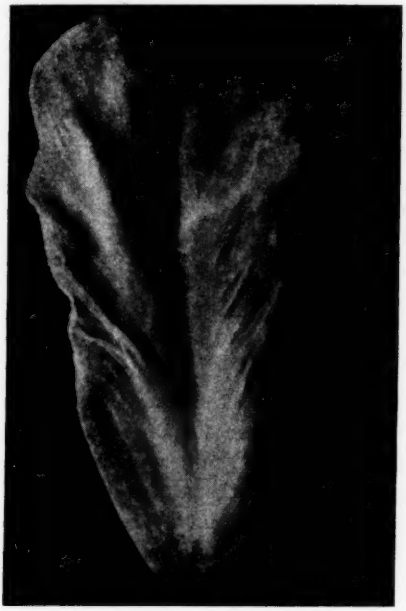


Fig. 2. Leaflet from a plant of line No. 21, showing a crisp or folded structure. (Slight magnification.) — Fig. 3. Profiles of successive sections cut perpendicularly to the foldings of a *cri cri* leaflet.

TABLE 1. *Scoring of the Cri—Gp segregation.*

A. The original F_2 data (self repulsion).

Cross No.	Symbol Genotype	a_1 *Cri Gp*	a_2 *Cri gp*	a_3 *cri Gp*	a_4 *cri gp*	*n*	germ. %
44/65	No. of plants	84	50	35	—	169	89
44/66	» » »	49	26	30	—	105	87
Total	No. of plants	133	76	65	—	274	88

B. Completing F_3 classification of 25 *Cri Gp* plants.

Cross No.	Symbol Genotype	*i* *Cri Gp* / *Cri Gp*	*j* *Cri Gp* / *cri Gp*	*k* *Cri Gp* / *Cri gp*	*l* *Cri Gp* / *cri gp*	*m* *Cri gp* / *cri Gp*	*n*
44/65 and 66	No. of fam.	—	3	3	—	10	25

C. The extra F_2 data from the 19 F_3 families of the category *m* above.

Pooled data.	Symbol Genotype	a_1 *Cri Gp*	a_2 *Cri gp*	a_3 *cri Gp*	a_4 *cri gp*	*n*	germ. %
	No. of plants	539	255	221	4	1019	89

As to the selection of the F_3 families, see the text.

ations, which indicate absolute linkage between *Cri* and *Gp* and are homogeneous, as can be seen from the analysis of χ^2 below.

Segregation	*Cri* : *cri*		*Gp* : *gp*		Abs. link. (2 : 1 : 1)	
	DF	χ^2	DF	χ^2	DF	χ^2
Cross No. 44/65	1	1,659	1	1,895	2	2,669
» » 44/66	1	0,714	1	0,003	2	0,771
Total	2	2,373	2	1,898	4	3,440
Deviation	1	0,238	1	0,095	2	1,117
Heterogeneity	1	2,135	1	0,803	2	2,323

In this case of strong linkage tested in repulsion crosses it would, however, be unwise not to extend the analysis to the F_3. For such an analysis seed was taken from F_2 plants of the *Cri Gp* genotype. Only plants giving at least 60 seeds were chosen, and the number of seeds sown per family was restricted to 60. The reason for this was purely practical, since in the trial fields at Alnarp peas are grown in 600 cm. long rows and I usually have 10 cm. between the plants within the rows. From the genetical point of view it would have been better to restrict the number of plants per family to 25 and grow a greater number of families. When allowance is made for a germinating power of 80 % this size of family would have given a sufficient number of plants for a reliable ($P = 0{,}01$) classification of the families (*cf.* MATHER, 1938, pp. 26—28).

On applying the method of maximum likelihood (*cf.* LAMM, 1947) the F_3 data of Table 1 B give the crossing-over value $11{,}4 \pm 4{,}32$ % between *Cri* and *Gp*. Besides additional F_2 information was obtained from the F_3 families belonging to the category *m* of Table 1 B. The figures obtained are given in Table 1 C. Here the single factor segregation with respect to *Gp* is very good ($\chi^2 = 0{,}095$; $P = 0{,}80$—$0{,}70$), whereas the segregation of *Cri* deviates slightly from expectation owing to shortage of *cri* plants ($\chi^2 = 4{,}632$; $P = 0{,}05$—$0{,}02$). For the estimation of linkage the product method would then perhaps be preferable to the method of maximum likelihood (*cf.* MATHER, 1938, p. 90). The former method gives the linkage value $13{,}52 \pm 3{,}06$ % whereas the latter gives $13{,}64 \pm 3{,}06$ %. Obviously the segregation of *Cri* is sufficiently good for the use of the maximum likelihood method of estimating linkage.

It seems perhaps astonishing that no single *cri gp* plant has been observed among the 274 original F_2 plants. The minimum size of family required in order to obtain at least one individual of that category is, however, with the maximum error 1 : 20, no less than 685 (*cf.* LAMM, 1947).

The final determination of the crossing-over value between *Cri* and *Gp* has been worked out in the combined estimation by means of the maximum likelihood method given in Table 2. This table gives an iteration solution of p and also allows of a determination of the heterogeneity (*cf.* MATHER, 1938, pp. 82—84). We may conclude that the combined F_2 and F_3 data are homogeneous, and that the joint estimate of the crossing-over value between *Cri* and *Gp* is $11{,}9 \pm 2{,}45$ %.

TABLE 2. *Combined estimate of the recombination fraction p between Cri and Gp from the data of Table 1 and proof of homogeneity.*

Max. likelihood	$p = 0{,}118$	$p = 0{,}119$	$p = 0{,}1188$	$\chi^2 = S(D^2 : I)$
A.				
$+ 2a_1 \times p : (2 + p^2)$	+ 15,585472	+ 15,715812	+ 15,689744	
$- 2(a_2 + a_3) \times p : (1 - p^2)$.	— 33,745812	— 34,039938	— 33,981282	
Total	— 18,160340	— 18,324126	— 18,291538 = D_A	2,0428
Diff.	+ 0,163786		+ 163,786 = I_A	
B.				
$+ 2(m-j-k) \times p : (2 + p^2)$	+ 63,162176	+ 63,690396	+ 63,584752	
$- (2m + j + k) : (1 - p)$ ..	— 113,922032	— 114,914968	— 114,716952	
$+ (j + k) : p$	+ 67,796608	+ 67,226888	+ 67,340000	
Total	+ 17,036752	+ 16,002316	+ 16,207800 = D_B	0,2539
Diff.	+ 1,034436		+ 1034,436 = I_B	
C.				
$+ 2a_1 \times p : (2 + p^2)$	+ 1,523392	+ 1,536132	+ 1,533584	
$- 2(a_2 + a_3) \times p : (1 - p^2)$.	— 49,886584	— 49,943212	— 49,931904	
$+ 2a_4 : p$	+ 50,847456	+ 50,420166	+ 50,505000	
Total	+ 2,484264	+ 2,013086	+ 2,106680 = D_C	0,0094
Diff.	+ 0,471178		+ 471,178 = I_C	
A + B + C.				
Grand total	+ 1,360676	— 0,308724		2,3061
Diff.	+ 1,669400			(DF = 2)

Joint estimate of $p = 0{,}1188 \pm 0{,}02447$ $\qquad s_p = \sqrt{1 : 1669{,}4} = \pm 0{,}02447$

As can be seen from Table 1 C, four *cri gp* plants have been found. These plants were rather weak and only one of them gave seed. The weakening effect of *gp* has been discussed by LAMPRECHT (1948 a). This effect is probably stressed in combination with *cri*.

The crosses 44/65 and 44/66 are also segregating with respect to the length factor *Le*. In the F_2 the following result was obtained.

Genotype	*Cri Le*	*Cri le*	*cri Le*	*cri le*	*n*	% germ.
44/65	105	29	27	8	169	89
44/66	57	18	19	11	105	87
Total	162	47	46	19	274	88

The analysis given below shows that there is free recombination between *Cri* and *Le*.

Segregation	*Cri* : *cri* DF	χ^2	*Le* : *le* DF	χ^2	Linkage DF	χ^2
Cross No. 44/65	1	1,659	1	0,870	1	0,053
» » 44/66	1	0,714	1	0,384	1	2,143
Total	2	2,373	2	1,254	2	2,187
Deviation	1	0,238	1	0,122	1	1,182
Heterogeneity	1	2,135	1	1,132	1	1,005

The linkage relation between *Cri* and *Wa* has been studied in the cross No. 42/28 between line No. 6 (*Cri wa*) and line No. 21 (*cri Wa*). The F_2 data of this cross are given below.

Genotype	*Cri Wa*	*Cri wa*	*cri Wa*	*cri wa*	*n*	% germ.
42/28	301	85	94	35	515	88

There is no indication of linkage between *Cri* and *Wa* (χ^2 *Cri* : *cri* = = 0,001; χ^2 *Wa* : *wa* = 0,793; χ^2 linkage = 1,346). In an earlier paper (LAMM, 1947) the linkage between *Wa* and *Gp* has been discussed. A crossing-over value of $39{,}5 \pm 5{,}92$ % was obtained. In view of the high standard error this linkage is rather uncertain. It was also suggested that *Wa* is probably situated in the neighbourhood of *Fs*. This hypothesis will be checked by a series of new crosses giving F_2 progenies in 1949. If the wax-factor *Wa* is really situated as suggested, then *Cri* is probably situated to the left of *Gp*, the order of the genes being *Cri—Gp—Wa*.

THE EXTRA RAPID TRANSLOCATION.

Linkage relations. — LAMPRECHT (1946) reported a new case of interchange between the *B*-chromosome and the *Gp*-chromosome, *i. e.* between chromosome III and V (LAMPRECHT, 1948 b). In this translocation, which seems to be that of Extra Rapid, the point of interchange is situated to the left of the segment *St—B* of the *B*-chromosome (LAMM,

1948). LAMPRECHT (1946) assumes that the point of interchange in the *Gp*-chromosome is probably located between *Fs* and *Ast*. As an average of a great number of investigated crosses LAMPRECHT (1948 b) has worked out the following map of the *Gp*-chromosome in connection with which it must be pointed out that the crossing-over values are highly variable:

$$—Cp—18{,}5\ \%—Gp—36{,}1\ \%—Fs—28{,}0\ \%—Ast—$$

From F_2 data given by LAMPRECHT (1946), LAMM (1948) found the linkage value $12{,}2 \pm 1{,}17$ % between *Tr* and *Fs*. On the basis of the linkage data relating to the *Gp*-chromosome then available I found that *Tr* could not be situated between *Fs* and *Ast*, which, as was pointed out by LAMPRECHT (1948 a), was a too hasty inference. The F_2 data obtained from NILSSON, mentioned in the introduction of this paper, have made it possible to calculate the linkage between *Tr* and *Gp*. Due to these calculations *Tr* is situated within the segment *Gp*—*Fs*.

NILSSON made a cross between his yellow-podded (*gp*) line No. 174 of standard structure and a line of the green-podded (*Gp*) variety Extra Rapid of deviating structural type. Besides three plants, which could not be classified, and six trisomic plants his F_2 gave the following segregation:

22 fertile *Gp* : 18 fertile *gp* : 45 semisterile *Gp* : 3 semisterile *gp*.

Applying the χ^2 formulae given by LAMM (1948) the following values are obtained: χ^2 fertility : semisterility $= 0{,}727$, χ^2 *Gp* : *gp* $= 0{,}061$, χ^2 linkage *Tr*—*Gp* $= 17{,}515$***. The linkage between *Tr* and *Gp* is thus highly significant and can be estimated either by the method of maximum likelihood (*cf.* LAMM, 1948) or by the product method (JOACHIM, 1947). Using the former method, a crossing-over value of $6{,}5 \pm 3{,}32$ % has been obtained.

The standard error 3,32 % has been calculated arithmetically from the iteration solution. It could also be obtained by a formula containing the mean amount of information given by JOACHIM (1947). Determined by this last method it amounts to 3,73 %. These two methods give slightly different results (*cf.* MATHER, 1938, pp. 72—73).

Unfortunately no three-point test has been made for *Gp*, *Tr* and *Fs*, but the available data indicate the following order: *Gp*—6,5 %—*Tr*—12,2 %—*Fs*. In most cases crossing-over near the point of interchange is reduced, and it is therefore not astonishing to find a considerable reduction of the *Gp*—*Fs* distance. We have seen that in standard lines

this distance is about 36 % with rather deviating single cross values (*cf.* LAMPRECHT, 1948 b). The reduction to 18,7 % (12,2 % + 6,5 %) seems to be of about the same order as in the translocation heterozygote between HAMMARLUND's K-line and the standard structural type. Here in F_2 progenies investigated by PELLEW (1940), besides trisomic plants, the following segregations were found:

28 fertile *Fs* : 14 fertile *fs* : 24 semisterile *Fs* : 4 semisterile *fs*
4 » *Gp* : 20 » *gp* : 25 » *Gp* : 1 » *gp*

A χ^2 analysis of these two progenies gives:
χ^2 fertility : semisterility = 2,800 and 0,080, χ^2 *Fs* : *fs* and *Gp* : *gp* = 0,019 and 7,707**, χ^2 linkage *Tr*—*Fs* and *Tr*—*Gp* = 3,219 and 40,560***.

In the first family χ^2 for linkage is not significant. A determination of the crossing-over value between *Tr* and *Fs*, however, gives the value 18,6 ± 7,7 %. In the second family the *Gp*-segregation is rather deviating from expectation, and from this point of view the linkage calculation, which gives less than 1 % crossing-over, is of little value. Obviously, however, the point of translocation, which may or may not be identical with that of Extra Rapid, is coupled with *Gp*.

According to SANSOME (1938), the structural types represented by HAMMARLUND's K-line and Extra Rapid should have no chromosome interchanged in common. The present investigations, however, have shown that the *Gp*-chromosome is involved in both types. The F_1 between these lines, which has not been cytologically examined, is expected to give a ring of six.

DISCUSSION.

The new gene *cri* of *Pisum* exerts a pleiotropic effect rather comparable with that known from the *compactum* type described by LAMPRECHT (1935) which is caused by the gene *brev*. Plants homozygous for *brev* are characterized by a compact branching in the leaf-axes and a very reduced female fertility. In *compactum* plants the sterility is diplontic and due to morphological abnormalities of the filaments and carpels. The reduced fertility caused by *cri* is also diplontic and probably due to the crisp or folded structure of the carpels.

LAMPRECHT (1935, 1939, 1945 a) is of the opinion that the *compactum* type should be regarded as belonging to the numerous so-called »complex mutations» described by him. In these mutations, which give monofactorial segregations, aberrant characters occur concomitantly in

vegetative and floral parts of the plants. As to the *compactum* type, LAMPRECHT (1939) suggests that this mutation is caused by a deficiency. In maize MCCLINTOCK (1944) has shown that monofactorially segregating recessive alleles may be caused by small homozygous deficiencies. In *Pisum* the deficiency hypothesis has not been checked by cytological investigations, and is thus still open to considerable doubt. With respect to my future *Pisum* investigations I am above all interested in *Cri* as being a fairly good marker gene situated in a chromosome of particular importance as regards the studies of the Extra Rapid and the HAMMARLUND's K-line translocation.

In *Pisum sativum* several spontaneous reciprocal translocations are known. One of the questions to be answered is whether they are distributed at random or whether they are perhaps localized to certain definite regions of the chromosomes. LAMPRECHT (1946) suggests that the appearance of semisterile plants in *Pisum* is due to crossing-over between small homologous segments of non-homologous chromosomes. In a discussion of the origin of *Pisum sativum* in the light of polymeric genes he assumes that this species has originated from tetraploid ancestors (LAMPRECHT, 1947). The duplications from this point of view should represent a residue of the previous complete tetraploidy. Probably duplications do exist in *Pisum*. This was suggested by DE HAAN (1932), and my own investigations of the *Cy*-factors (LAMM, 1937 and 1947) are designed to test the validity of these suggestions. In order to explain the occurrence of duplications or repeats in a diploid organism it is, however, not necessary to assume this rather improbable polyploid origin.

Workers on *Drosophila* (DOBZHANSKY, 1941) and *Sciara* (METZ, 1947) have pointed out the importance of repeat formation in evolution, since apart from polyploidy this is the only known process which may lead to an increase of the number of genes in the germ plasm of the organism. SUTTON (1937) has reported an interesting case detected by SANSOME, where crosses between *Pisum sativum* and an inbred line of *Pisum humile* revealed a complicated double translocation system from which chromosomes with duplicated end-segments had been derived.

As a special case supporting his hypothesis concerning the distribution of translocations LAMPRECHT (1947) refers to the reciprocal translocation discussed in this paper. He claims that »it could gene-analytically be proved that the breaking of the chromosomes at the interchange took place just at the point where the two polymeric genes *F* and *Fs* are placed in the chromosome in question». LAMM (1948),

however, has pointed out that the crossing-over value between *Tr* and *Fs* is $12{,}2 \pm 1{,}17$ %, which, if due consideration is taken to a probable suppression of crossing-over, may mean a considerable distance. Nevertheless further investigations will perhaps show that the points of interchange are really situated within duplicated segments, but, even if this should happen to be true, certain alternative hypotheses as to the primary causes of this assumed localization may be at least as plausible as that given by LAMPRECHT. With the present knowledge of the cytology and genetics of *Pisum* a valid theory concerning the distribution of translocations could not be proposed.

As regards the translocation heterozygote between Extra Rapid and the standard structural type, suppression of crossing-over was strong in the *St—B* segment of the *B*-chromosome (LAMM, 1948). This segment would then probably belong to the chromosome arm of *Tr*, since in translocation heterozygotes suppression of crossing-over is confined to the arms interchanged, whereas crossing-over in the arms not involved is usually normal or slightly increased (*cf.* LUDWIG, 1938, p. 137). It is then improbable that the centromere is situated between *Tr* and *St*. Further studies of the crossing-over values between a number of genes of the *B*- and *Gp*-chromosomes in translocation heterozygotes between Extra Rapid and the standard structural type may perhaps give a valuable contribution to the question of the localization of the centromeres on the linkage maps of these chromosomes.

I wish to express my gratitude to the Horticultural Research Station at Alnarp for granting me the loan of experimental grounds and of manual help, and address these thanks to the Head of the Institution, Professor F. NILSSON. I am also indebted to my colleague in *Pisum* investigations Mr. E. NILSSON for the valuable data that he has put at my disposal.

SUMMARY.

In the present *Pisum* investigation the following main results have been obtained.

(1) A new gene *Cri* is presented. Characters of plants homozygous for the recessive allele *cri* are described.

(2) The factor *Cri* is linked with *Gp*. The crossing-over between *Cri* and *Gp* has been estimated at $11{,}9 \pm 2{,}45$ %.

(3) A contribution is made to the knowledge of the Extra Rapid translocation. In an earlier paper I showed that the point of interchange was situated to the left of the segment *St—B* of the *B*-chromosome. In the present paper it is shown that this point in the other chromosome involved, *i. e.* the *Gp*-chromosome, is situated within the segment *Gp—Fs*.

(4) The *Gp*-chromosome is involved in both the Extra Rapid structural type and in that of HAMMARLUND's K-line.

LITERATURE CITED.

1. DOBZHANSKY, T. 1941. Genetics and the origin of species. Second edition. — Columbia Univ. Press, New York.
2. DE HAAN, H. 1932. The heredity of emergences in *Pisum sativum*. — Genetica XIV: 319—320.
3. JOACHIM, G. S. 1947. The product method of calculating linkage from F_2 data involving semisterility; and its application to a barley translocation. — Genetics, Vol. 32: 580—591.
4. LAMM, R. 1937. Length factors in dwarf peas. — Hereditas XXIII: 38—48.
5. — 1947. Studies on linkage relations of the *Cy* factors in *Pisum*. — Hereditas XXXIII: 405—419.
6. — 1948. Linkage values in an interchange complex in *Pisum*. — Hereditas XXXIV: 280—288.
7. LAMPRECHT, H. 1935. Eine *Pisum*-Form mit *compactum*-Verzweigung und verkürzten Staubfäden. — Hereditas XX: 94—102.
8. — 1939. Über Blüten- und Komplex-Mutationen bei *Pisum*. — Zeitschr. f. ind. Abstamm.- u. Vererb.-Lehre, Bd. LXXVII: 177—185.
9. — 1945 a. Durch Komplexmutationen bedingte Sterilität und ihre Vererbung. — Arch. der Julius Klaus-Stiftung f. Vererbungsforsch., Sozialanthr. u. Rassenhygiene, Ergänzungsbd. zu Bd. XX: 126—141.
10. — 1945 b. Intra- and interspecific genes. — Agri Hortique Genetica III: 45—60. (Landskrona, Sweden.)
11. — 1946. Die Koppelungsgruppe *Uni—M—Mp—F—St—B—Gl* von *Pisum*. — Agri Hortique Genetica IV: 15—42.
12. — 1947. The inheritance of the number of flowers per inflorescence and the origin of *Pisum*, illustrated by polymeric genes. — Agri Hortique Genetica V: 16—25.
13. — 1948 a. Further studies of the linkage group *Cp—Gp—Fs—Ast* of *Pisum sativum*. — Agri Hortique Genetica VI: 1—9.
14. — 1948 b. The variation of linkage and the course of crossing over. — Agri Hortique Genetica VI: 10—48.
15. LUDWIG, W. 1938. Faktorenkoppelung und Faktorenaustausch bei normalem und aberrantem Chromosomenbestand. — Georg Thiemes Verlag, Leipzig.
16. MATHER, K. 1938. The measurement of linkage in heredity. — Methuen's Monographs on biological subjects. Methuen & Co. Ltd. London.

17. McClintock, B. 1944. The relation of homozygous deficiencies to mutations and allelic series in maize. — Genetics, Vol. 29: 478—502.
18. Metz, C. W. 1947. Duplication of chromosome parts as a factor in evolution. — The Am. Nat., Vol. LXXXI: 81—103.
19. Pellew, C. 1940. Genetical studies on the first reciprocal translocation found in *Pisum sativum*. — Journal of Genetics, Vol. XXXIX: 363—390.
20. Sansome, E. 1938. Segmental interchange lines in *Pisum sativum*. — Nature, Vol. 142: 674—675.
21. Sutton, E. 1937. Cytological studies of *Pisum*. I. Structural hybridity in *P. humile*. — Annals of Bot., New Series, Vol. I: 785—796.

CONTENTS.

Introduction 203
The gene *Cri* 203
Origin and description 203
Linkage relations 204
The Extra Rapid translocation 208
Linkage relations 208
Discussion 210
Summary 212
Literature cited 213

EXPERIMENTAL STUDIES IN SCANDINAVIAN ALPINE PLANTS

I. ECOTYPICAL DIFFERENTIATION IN MELANDRIUM ANGUSTIFLORUM (RUPRECHT) WALPERS

BY *AXEL NYGREN*
ROYAL AGRICULTURAL COLLEGE OF SWEDEN, UPSALA

THE alpine plant *Melandrium affine* J. VAHL or, as it is now said to be named, *M. furcatum* (RAF.) HULTÉN (HULTÉN, 1944, p. 702) forms an arctic circumpolar collective species, the European and western Siberian forms of which are usually brought together as *M. angustiflorum* (RUPRECHT) WALPERS (HYLANDER, 1945, pp. 161—162). HULTÉN (*l. c.*) proposes the name *M. furcatum* (RAF.) HULTÉN ssp. *angustiflorum* (RUPR.) HULTÉN. In Scandinavia the species mentioned is very rare, but is, in spite of this, known from Sweden and Norway as well as from Finland (*cf.* BJÖRKMAN, 1947, p. 310).

In the summer of 1946 the present writer collected seeds of *M. angustiflorum* in the Alta district of northern Norway (70° N. lat.) and at Björkliden in the Swedish Lapland (68°30′ N. lat.). In Alta the species occurred very rarely at Raipas on gravel-banks along the river Alta about three miles east of its outflow into the Alta fiord. The locality is situated between five and ten metres above the sea-level. The locality at Björkliden is classical to Sweden. *Melandrium* here grows on solifluxion banks near the northern mouth of the Nuolja tunnel. In the same locality the species is also growing along the very bed of the Riksgränsen Railway. The place lies about 350 metres above the sea-level, and the distance to the Atlantic is some twenty miles.

The collected seeds had a high germinating percentage, and in the summer of 1947 there were in cultivation about thirty Alta plants and twice as many from Björkliden. The first year the plants formed rosettes only, which, however, showed obvious differences respecting the breadth of leaves in the two collections. In the autumn of 1947 specimens from both localities were planted out together in rows. All plants survived the winter of 1947—48. Those originating from Björkliden began to flower on the 12th of May, while the Alta type did not

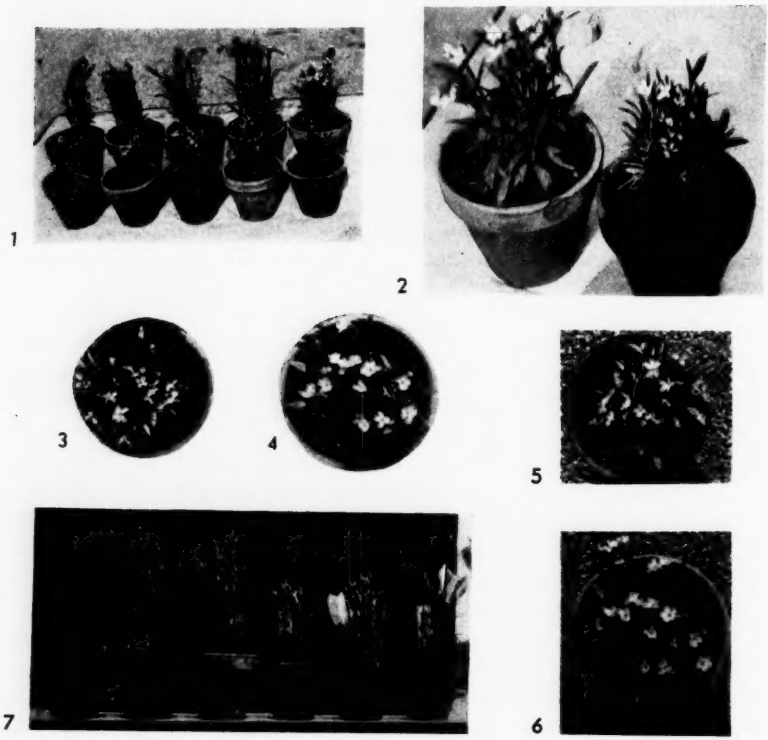


Fig. 1. In the first row five plants from Alta, in the second row five plants from Björkliden. — Fig. 2. To the left one plant from Björkliden, to the right one from Alta. — Fig. 3. The Alta type from above. — Fig. 4. The Björkliden type from above. — Fig. 5. The same as No. 3. — Fig. 6. The same as No. 4. — Fig. 7. To the left three plants from Björkliden, to the right three from Alta.

start its anthesis until the 26th of the same month. Fig. 1 was taken on the 29th of May; here the flowering is almost finished in four plants from Björkliden, while it is still in full progress in the Alta specimens. The morphological differences between the two types are striking (Fig. 2). In the Björkliden form the petals are broadest at the apex and slightly nipped out, while in the Alta type they are of about the same breadth at the base as at the apex and have a deep incision in the latter (*cf.* Figs. 3 and 5 with Figs. 4 and 6). In the Alta plants the corollas are purely white, but in those from Björkliden the petals are white with a reddish-violet back. The capsule is rounded-oval in all

TABLE 1. *Measurements of different morphological characters in Melandrium angustiflorum from Björkliden and Alta, respectively.*

Plant number	Stalks		Leaves			Flowers
	Height	Number of branches	Length	Breadth	Rel. length/ breadth	Number of flowers/branch
B. 1 ...	18,5	18	2,9	0,54	5,37	2,78
B. 2 ...	20,2	18	3,5	0,72	4,86	3,00
B. 3 ...	19,1	9	3,1	0,48	6,45	2,11
B. 4 ...	22,0	17	3,9	0,61	6,39	3,35
B. 5 ...	15,0	5	2,4	0,41	5,85	2,00
B. 6 ...	19,0	17	3,5	0,65	5,38	2,76
B. 7 ...	13,8	13	2,0	0,48	4,17	1,85
B. 8 ...	16,7	23	2,7	0,59	4,58	2,30
B. 9 ...	18,0	17	3,0	0,50	6,00	2,00
B. 10 ...	20,5	16	3,6	0,71	5,07	2,60
B. 11 ...	10,9	12	1,6	0,38	4,21	1,83
B. 12 ...	16,8	16	3,1	0,55	5,64	2,69
B. 13 ...	19,3	19	2,7	0,53	5,09	2,32
B. 14 ...	10,5	9	2,2	0,60	3,67	1,57
B. 15 ...	23,7	8	4,0	0,68	5,88	2,25
B. 16 ...	13,5	9	1,8	0,40	4,50	2,40
B. 17 ...	10,0	9	1,8	0,45	4,00	1,33
B. 18 ...	19,1	12	3,9	0,70	5,58	2,58
B. 19 ...	18,8	18	3,2	0,60	5,33	2,39
B. 20 ...	13,5	18	2,0	0,49	4,08	2,11
B. 21 ...	10,4	4	1,6	0,35	4,57	2,25
B. 22 ...	17,3	10	3,8	0,69	5,51	2,43
B. 23 ...	19,4	20	2,8	0,60	4,67	2,35
B. 24 ...	19,0	15	3,6	0,68	5,30	2,39
B. 25 ...	14,7	9	1,9	0,48	3,95	2,00
B. 26 ...	13,5	3	3,2	0,75	4,27	2,00
M.... .	16,7	13,2	$2,84 \pm 0,1526$	$0,56 \pm 0,0212$	$5,07 \pm 0,1585$	$2,80 \pm 0,0862$
A. 1 ...	13,1	16	3,0	0,32	9,38	1,56
A. 2 ...	12,5	8	1,6	0,25	6,40	1,25
A. 3 ...	13,8	24	2,7	0,32	8,44	1,41
A. 4 ...	9,3	2	2,6	0,30	8,67	1,00
A. 5 ...	19,5	7	3,5	0,33	10,61	1,00
A. 6 ...	11,8	9	1,8	0,25	7,20	1,00
A. 7 ...	15,2	6	2,2	0,25	8,80	1,67
A. 8 ...	10,8	4	1,8	0,21	8,57	1,10
A. 9 ...	16,1	14	2,0	0,25	9,09	1,36
A. 10 ...	17,3	28	2,0	0,33	8,79	1,54
A. 11 ...	15,5	17	2,9	0,28	10,36	1,65
A. 12 ...	14,5	12	3,1	0,34	9,12	1,00
A. 13 ...	17,2	16	2,7	0,34	7,94	1,50
A. 14 ...	19,5	7	4,0	0,39	10,26	1,43
A. 15 ...	16,9	13	2,6	0,24	10,83	1,62

Plant number	Stalks		Leaves			Flowers
	Height	Number of branches	Length	Breadth	Rel. length/ breadth	Number of flowers/branch
A. 16 ...	9,3	3	1,5	0,19	7,89	0,75
A. 17 ...	17,5	17	2,7	0,28	9,64	1,76
A. 18 ...	16,1	15	2,9	0,32	9,06	1,87
A. 19 ...	17,8	22	3,7	0,43	8,60	1,82
A. 20 ...	11,9	5	1,6	0,23	6,96	1,60
A. 21 ...	14,6	2	2,7	0,35	7,71	1,50
M.....	14,8	11,8	$2{,}60 \pm 0{,}1520$	$0{,}29 \pm 0{,}0135$	$8{,}97 \pm 0{,}9548$	$1{,}40 \pm 0{,}0855$
B/A			$t = 1{,}1142$ ($P = 0{,}3$)	$t = 10{,}7441^{***}$	$t = 4{,}1631^{***}$	$t = 11{,}5321^{***}$

All measures in cm. A = Alta. B = Björkliden. Every figure is based on ten measurements of the respective character. All measurements are made on plants in ripe fruit-stages.

the Björkliden specimens, while the Alta type has long, slender capsules. In both groups it is densely pilose. The number of stalks on the plants varies greatly in both cases; the Björkliden form, however, is the more branched one and hence this form has more flowers than that from Alta. A statistical analysis of the material shows (see Table 1) that on an average the Björkliden plants have developed $2{,}80 \pm 0{,}0862$ flowers a stalk, but those of Alta $1{,}40 \pm 0{,}0855$. The difference is significant, which is clear from the *t*-value $11{,}5321^{***}$ (47 degrees of freedom). The rosette-leaves in 1948 retained the difference of 1947, as can be seen from Fig. 2. In the Björkliden form the leaves average $2{,}84 \pm 0{,}1526$ cm. long and $0{,}56 \pm 0{,}0212$ cm. broad, while the figures of the Alta type are $2{,}60 \pm 0{,}1520$ cm. for the length and $0{,}29 \pm 0{,}0135$ cm. for the breadth. The difference in leaf-length is not significant ($t = 1{,}1142$), but that of the breadth is statistically significant, the *t*-value being $10{,}7441^{***}$. The figures for the relation leaf-length/leaf-breadth are $5{,}07 \pm 0{,}1585$ in the Björkliden plants and $8{,}97 \pm 0{,}9548$ in those from Alta. The difference is significant ($t = 4{,}1631^{***}$). The hairiness of the rosette-leaves is diverse in the two types. An estimation of all plants included in the investigation gave as an average the figure 34,2 for the Björkliden material and 6,9 for the Alta.

The seed-setting is about the same in both types. *Melandrium angustiflorum* is an autogamous species, which has a perfect formation of seeds even after bagging. The germinability of the seeds lies between 90 and 100 per cent, if they are sown out immediately after ripening. Both forms are fully fertile in crosses with each other in both directions. The germination of seeds obtained after crossing seems to be somewhat

reduced, but the number of crosses made is too small to permit of a statistical analysis. The two forms obviously belong to the same *eco-species* (TURESSON, 1922 and 1929, p. 332). The chromosome number of both forms is $2n = 48$, and both have a regular meiosis on the male as well as on the female side. It has not been possible to cross the types either with *Melandrium rubrum* ($2n = 24$) from Alta or with *M. apetalum* ($2n = 24$) from Dovre in Norway and Abisko in Sweden. Numerous crosses have been made in both directions. Obviously there is a crossing-barrier between *M. angustiflorum* and the other two species mentioned, probably depending on differences in the chromosome number.

From a morphological or taxonomical point of view the two types of *M. angustiflorum* from Björkliden and Alta, respectively, are so different that they deserve the rank of subspecies as well as, for instance, the various races of the species *Papaver radicatum* coll. in Scandinavia, which have been distinguished by NORDHAGEN (1931). These races are easy to cross, in spite of differences in the chromosome number in some cases, not only within themselves, but also with the garden type of *Papaver nudicaule* (NYGREN, unpublished).

Much more important than a taxonomical classification on exterior characters of the two *Melandrium* forms in question is, however, their value from a genetical and ecological point of view. The plants from Björkliden originate from the population which since many years ago has been known from the slopes of Mount Nuolja. Apparently the species thrives on the open gravel-strips of the Riksgränsen Railway and is going to spread along these. From the beginning, however, the population belongs to the mountains, where *Melandrium angustiflorum* grows more especially on solifluxion tongues along the slopes just in the same localities as *M. affine* J. VAHL occurs in Greenland (SØRENSEN, 1933, p. 28). In northern Norway *M. angustiflorum* is only known from the gravel-banks of the rivers Alta and Raipas (LID, 1944, p. 216). In spite of the fact that these localities seem to be secondary ones to a Swedish observer who knows the plant from Torne Lappmark in northern Sweden, there is but little probability of the species occurring on the slopes of the mountains around the two rivers just mentioned and from there now and then being carried down with the water to the banks. Northern Norway is in fact botanically well investigated, and *M. angustiflorum* is a fastidious species that requires specific localities, where as a rule it is easy to be found. Most probably the Alta type is an *alpine lowland ecotype* of *M. angustiflorum*, while the pop-

ulation at Björkliden is to be regarded as a *mountain ecotype* of the same species (*cf.* TURESSON, 1925, p. 189 for *Melandrium rubrum*). The localities of *M. angustiflorum* found by BJÖRKMAN (1947) in the Muddos district of Lule Lappmark in northern Sweden may possibly be Swedish analogues to that of Alta. The interpretation of the two forms as being different ecotypes also agrees with the time they begin to flower. The mountain ecotype, which at Björkliden grows 350 metres above the sea-level, flowers earlier than the lowland ecotype from Raipas near the sea-level, in spite of the fact that the former has been collected nearly one and a half latitudes further to the south than the latter. The reason why the morphological differences between the two ecotypes are so striking most likely depends on the magnitude of the populations. At Björkliden *Melandrium* is common here and there, a circumstance that gives the species chances to crossbreed. At Raipas in the Alta district it is very scanty, however. Here, only solitary specimens could be found several hundred metres separated from each other; in all, five plants were observed. Some causes of the sparse occurrence of the species in this locality may be inundations and rotatory ice-pressure.

Financial support has been received from Statens naturvetenskapliga forskningsråd.

LITERATURE CITED.

1. BJÖRKMAN, S. O. 1947. *Melandrium angustiflorum* (RUPR.) WALPERS anträffad i Muddos nationalpark. — Sv. Bot. Tidskr., Bd. 41, pp. 309—311.
2. HULTÉN, E. 1944. Flora of Alaska and Yukon. Pars IV. — Lunds Univ. Årsskr. N. F. Avd. 2. Bd. 40. Nr. 1, pp. 571—795.
3. HYLANDER, N. 1945. Nomenklatorische und systematische Studien über nordische Gefässpflanzen. — Uppsala Univ. Årsskr. 1945: 7, 337 pp.
4. LID, J. 1944. Norsk flora. — Oslo. 637 pp.
5. NORDHAGEN, R. 1931. Studien über die skandinavischen Rassen des *Papaver radicatum* ROTTB. sowie einige mit denselben verwechselte neue Arten. Vorläufige Mitteilung. — Bergens Mus. Årbok 1931. Naturvet. Rekke, nr. 2. 50 pp.
6. SØRENSEN, T. 1933. The vascular plants of East Greenland from 71°00 N. lat. to 73°30′ N. lat. — Meddelelser om Grønland, Bd. 101, Nr. 3. 175 pp. + 20 pl.
7. TURESSON, G. 1922. The species and the variety as ecological units. — Hereditas III, pp. 100—113.
8. — 1925. The plant species in relation to habitat and climate. — Hereditas VI, pp. 147—236.
9. — 1929. Zur Natur und Begrenzung der Arteinheiten. — Hereditas XII, pp. 323—334.

ON THE BEHAVIOUR OF UNIVALENTS AT MEIOSIS IN SOME INTERSPECIFIC GEUM HYBRIDS

BY *W. GAJEWSKI*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

DURING my stay at the Institute of Genetics in Lund I have made some observations on meiotical divisions in PMC's of different interspecific *Geum* hybrids. The differences in the behaviour of the univalents among the hybrids studied were so striking and peculiar that I have got the idea it would be worth describing them. The question why the univalents divide at the first meiotical division in some hybrids and do not do so in others is still quite obscure. Further, the rules governing their movements during meta- and ana-phase, orientation and splitting are far from being understood, although in the cytogenetic literature there are plenty of descriptions of very interesting differences in their behaviour. A more accurate knowledge of these very complicated phenomena could no doubt throw some new light on the question of the mechanics of chromosomal movements at meiosis.

MATERIAL AND METHODS.

The floral buds were fixed in the spring of 1948 from interspecific *Geum* hybrids cultivated on the experimental plots of the Botanical Garden, University of Warszaw, Poland. The meiosis of the following hybrids will be described here:

(1) *Geum canadense* JACQ. (21) × *Geum rivale* L. (21).
(2) *Geum montanum* L. (14) × *Geum rivale* L. (21).
(3) *Geum Quellyon* SW. (35) × *Geum rivale* L. (21).
(4) *Geum pyrenaicum* WILLD. (42) × *Geum rivale* (21).
(5) *Geum macrophyllum* WILLD. (21) × *Geum rivale* L. (21).

Floral buds were prefixed in CARNOY for 2—5 minutes and then fixed in KARPETCHENKO. They were embedded in paraffin and cut 15 μ thick. Permanent preparations were stained with cristal-violet according to NEWTON's method. The drawings were made with a camera lucida at the magnification of × 1700 and the microphotographs have a magnification of × 2800.

THE DESCRIPTION OF MEIOSIS WITH SPECIAL REFERENCE TO THE BEHAVIOUR OF THE UNIVALENTS.

For many years I have been studying the cytogenetic relationships in the genus *Geum* L., and the results will soon be published. There, the morphological descriptions of the species and hybrids, their fertility and the inheritance of specific traits will be found. Now, only some peculiarities of meiotic divisions in PMC's from a few selected hybrids will be described.

All the hybrids considered here were chosen in such a way that the pollinating parent was one and the same species, *Geum rivale* L., while the other, the pistillate, parent was different in every cross, representing a different degree of ploidity or a different degree of pairing with the chromosomes of *G. rivale*. The hybrids listed above form a series with increasing number of univalents. Probably, the causes of the incomplete conjugation are not the same for all five hybrids. In hybrids Nos. 2, 3 and 4 the number of bivalents corresponds in most of PMC's to the number of the chromosomes of the parent with a lower degree of ploidity and the rest of the chromosomes remain unpaired. In the case of hybrids 1 and 5, where both parents have the same chromosome numbers, the causes of the formation and the number of univalents are not so obvious and easy to explain. The differences in chromosomal conjugation between hybrids 1 and 5 are probably due to different degrees of chromosomal homology and to some genic influences causing asynapsis or precocious desynapsis. As a general rule it may be stated that even for one and the same hybrid the degree of chromosomal conjugation is more or less fluctuating, as can be seen from the following table:

Hybrid No. 1	No. of univalents	0	2	4	6						
	No. of PMC's	28	8	4	3					$n = 43$	M = 1,1
Hybrid No. 2	No. of univalents	5	7	9	11						
	No. of PMC's	2	60	7	2					$n = 71$	M = 7,25
Hybrid No. 3	No. of univalents	14	16	18	20	22					
	No. of PMC's	14	6	7	2	2				$n = 31$	M = 16,6
Hybrid No. 4	No. of univalents	19	21	23	25						
	No. of PMC's	2	35	8	6					$n = 51$	M = 21,7
Hybrid No. 5	No. of univalents	28	30	32	34	36	38	40	42		
	No. of PMC's	1	5	2	6	5	4	5	3	$n = 31$	M = 35,6

Not only is the number of univalents increasing absolutely but also in relation to the total number of the chromosomes:

Hybrid No.	Mean No. of univ.	Total No. of chrom.	Per cent of univ.	Most common configurat.
1	1,1	42	2,6	21_{II}
2	7,25	35	20,7	$14_{II} + 7_{I}$
3	16,6	56	29,6	$21_{II} + 14_{I}$
4	21,7	63	34,4	$21_{II} + 21_{I}$
5	35,8	42	85,2	$4_{II} + 34_{I}$

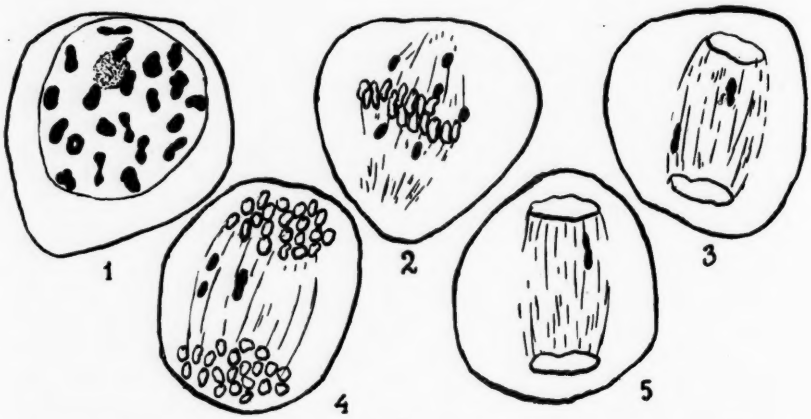
In all parental species the size and morphology of the somatic chromosomes are very similar. The length varies from 0,8 μ to 2,5 μ. In all parental species the course of meiosis is quite normal with no polyvalents and only rarely with some few univalents. The bivalents are nearly spherical or slightly elongated.

The early prophasal stages of meiosis in PMC's are difficult to study and a complete analysis of whole nuclei is usually impossible until diakinesis or, rarely, at late diplotene. At diakinesis in hybrids 1 and 5 the number of bivalents seems to be somewhat higher than in I-M. For instance, in hybrid 1 at I-M the percentage of cells with only bivalents is 65,1 (28 of 43), but in diakinesis it is 89,0 (18 of 21). Similar observations were made in hybrid 5. In diakinesis, besides bivalents of spherical or elongated shape with both chromosomes tightly synapsed, some loose bivalents with both partners connected end-to-end are to be found (Figs. 1 and 38). It seems very probable that these bivalents separate before metaphase and cause, at least in part, the observed difference in the number of univalents in diakinesis and metaphase. As in all *Geum* material there exists a pronounced tendency of the univalents and bivalents to appear in groups of two or to form chains consisting of many bodies, it is often very difficult to decide whether a real bivalent is concerned or only two univalents lying close together.

At the stage intermediate between diakinesis and metaphase, after the disappearance of the nuclear membrane, the chromosomes are more or less clumped together. This stage is of short duration and soon after the spindle is formed the chromosomes begin to form a metaphasal plate. The further course of meiosis beginning from prometaphase is quite dissimilar in different hybrids and must be described separately for any of them.

Hybrid No. 1 [*G. canadense* (21) $\times$ *G. rivale* (21)]. — In PMC's with only bivalents the course of meiosis is quite normal (Fig. 12). In PMC's with 2 to 6 univalents, the univalents always lie outside the equatorial plate, scattered on the spindle (Figs. 2, 13). They could never be found inside the plate or on its periphery at the same level as the

bivalents. At anaphase the synapsed chromosomes separate and move to opposite poles. As at late anaphase the number of PMC's with univalents lagging on the spindle between the two anaphasal groups is much lower than the number of PMC's with univalents at I-M, it must be concluded that the majority of the univalents pass to the poles at anaphase together with the bivalent halves. For instance: at I-M in 43 PMC's I have found 28 with 0_I, 8 with 2_I, 4 with 4_I, 3 with 6_I; at I-A in 108 PMC's I have found 3 cells with 1 and 2 with 2 lagging univalents.



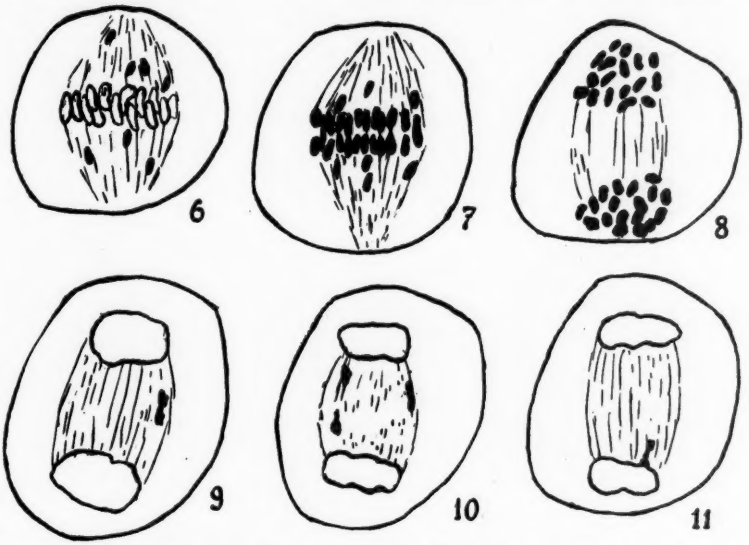
Figs. 1—5. Meiosis in hybrid No. 1. — Fig. 1. Diakinesis with loose bivalents. — Fig. 2. I-M with 6 univalents. — Figs. 3—5. I-A with dividing and stretched univalents.

The fact that the univalents usually pass undivided indicates the number of the chromosomes in both anaphasal groups, which was never found to be higher than 42.

At late anaphase the rare univalents that rest on the spindle are to be found on the equatorial plane. At this time they begin to elongate and have a peculiar shape of two united beads (Figs. 3—5). These two beads begin to move to the opposite poles and the chromosomal strand uniting them breaks or is elongated to a long, thin thread (Fig. 14). Often, such elongated univalents pass as a whole to one of the daughter nuclei (Figs. 3, 5). This peculiar behaviour of univalents is much more typical of the hybrids Nos. 3 and 4 and will be described here in greater detail. The division or stretching of the univalents and the subsequent movements of the latter are always much delayed, so that

when they reach the poles the nuclei have often already undergone telophasal changes. Such univalents rest in cytoplasm and are usually absorbed or, very rarely, form small micronuclei. The second meiotical division is rather normal. About 50 % of the pollen are poor.

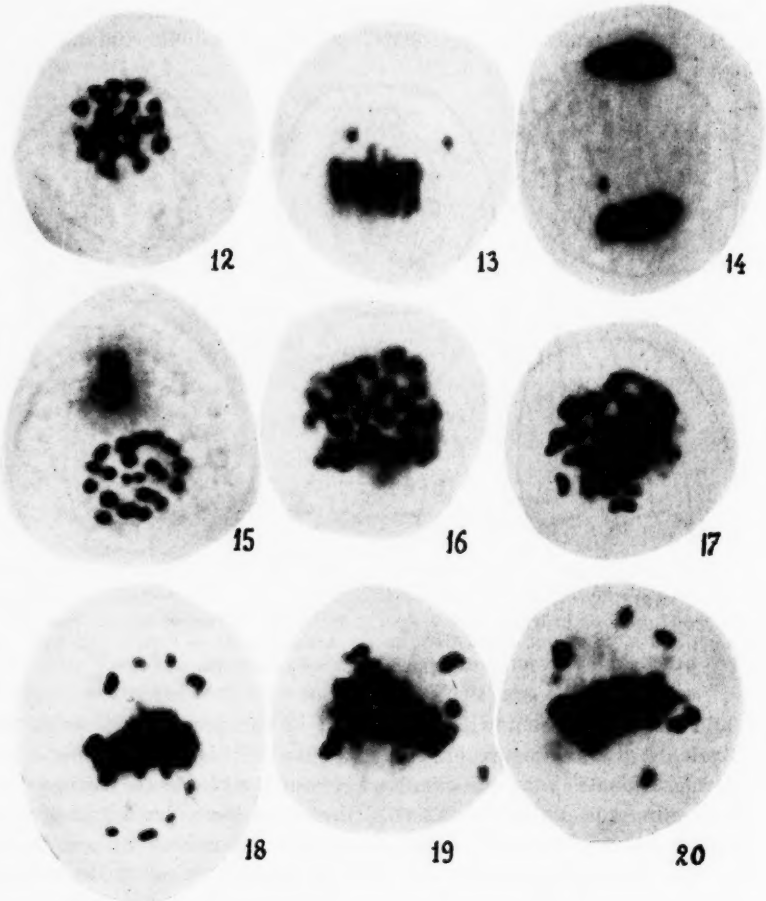
Hybrid No. 2 [*G. montanum* (14) × *G. rivale* (21)]. — The course of meiosis in this hybrid is similar to that described above in hybrid No. 1. In the great majority of PMC's the number of the univalents is



Figs. 6—11. Meiosis in hybrid No. 2. — Fig. 6. I-M with 7 univalents. — Fig. 7. Beginning of I-A. — Fig. 8. I-A with 17 a. 18 chromosomes. — Figs. 9—11. Late I-A with dividing and stretched univalents.

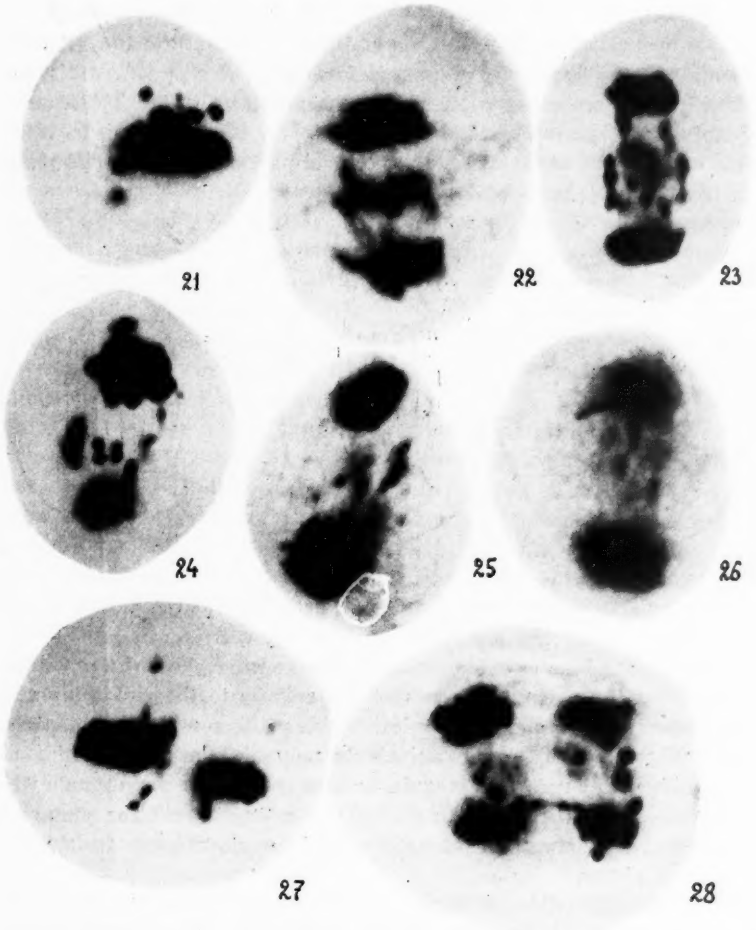
7 and in the rest somewhat higher. As in the preceding hybrid, the univalents at I-M are scattered on the spindle (Fig. 6). In some cases a few univalents lie on the equator between the bivalents. During anaphase numerous univalents are distributed to the poles together with bivalent halves (Fig. 7). Although at I-M the univalents are in all PMC's at late anaphase I have found univalents lagging on the spindle only in 32 % of PMC's and always in a number lower than at I-M (usually from 1 to 6). These univalents move to the equator and after a great delay begin to divide. Here, too, I have observed the peculiar stretching of the univalents, after which most of them pass as wholes to one of the nuclei and only few separate into two halves, going to

opposite poles (Figs. 9—11). The sum of the chromosomes in both anaphasal groups is usually 35 (Fig. 8) and the distribution of the chromosomes to both nuclei indicates a random assortment of the univalents:



Figs. 12—20. Meiosis in hybrid No. 1 (Figs. 12—15) and hybrid No. 4 (Figs. 16—20). — Fig. 12. I-M plate with 21 bivalents. — Fig. 13. I-M with 2 univalents. — Fig. 14. I-A with a stretched univalent. — Fig. 15. II-M with 22 chromosomes. — Figs. 16—17. I-M with univalents on the periphery of the plate. — Fig. 18. Early I-M with univalents on the spindle. — Figs. 19—20. I-M later stage.

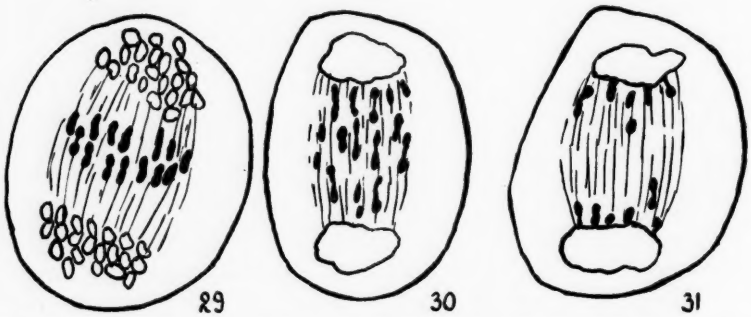
Chromosome numbers in I-A groups:	17 a. 18	16 a. 19	15 a. 20	14 a. 21	χ^2	P(3)
Number of PMC's found:	19	7	3	—		
Number of PMC's calculated:	16	9,5	3,1	0,4	1,623	0,658



Figs. 21—28. Meiosis in hybrid No. 4. — Fig. 21. Late I-M. — Fig. 22. Anaphasal ring of univalents. — Figs. 23—25. Late anaphase with divided and stretched univalents. — Fig. 26. Stretched univalent near the anaphasal nucleus (upper). — Fig. 27. II-M with chromosomes outside the plates. — Fig. 28. II-A with lagging chromosomes and a bridge from I-A.

The sum of the chromosomes in both anaphasal groups is rarely greater than 35, indicating that one or more univalents were divided into two chromatids. The second meiotical division is rather normal. There are about 70 % poor pollen grains.

Hybrid No. 3 [*G. Quellyon* (35) × *G. rivale* (21)]. — Here, the course of meiosis is quite different. At early metaphase the bivalents orientate at the equatorial plane and form a regular plate. At this time the univalents are scattered on the whole spindle (Fig. 41). The number of univalents is usually 14, but often higher. It seems here as if the plate of bivalents occupies only the central part of the equatorial plane, leaving the peripheral part free.



Figs. 29—31. Meiosis in hybrid No. 3. — Fig. 29. I-A with 14 univalents in a ring. — Figs. 30—31. Late I-A with divided and stretched univalents.

When the bivalents have already formed the plate, the univalents move from the peripheral parts of the spindle to the equator and in late metaphase form a more or less regular ring round the metaphasal plate (Figs. 42—44). Some univalents may also be found inside the plate. They are not quite regularly arranged, some being somewhat below or above the plate. In any case, in this hybrid the picture at the end of metaphase is quite different from that in the two hybrids described above.

At anaphase this difference is still more striking. The bivalents separate and the halves move to the poles. All the univalents rest on the equator, forming a more or less regular ring (Figs. 29, 45—52). Thus, at anaphase three distinct groups of chromosomes are to be found: on the poles the two anaphasal groups of bivalent halves and on the equator a ring or incomplete plate of univalents. The numbers

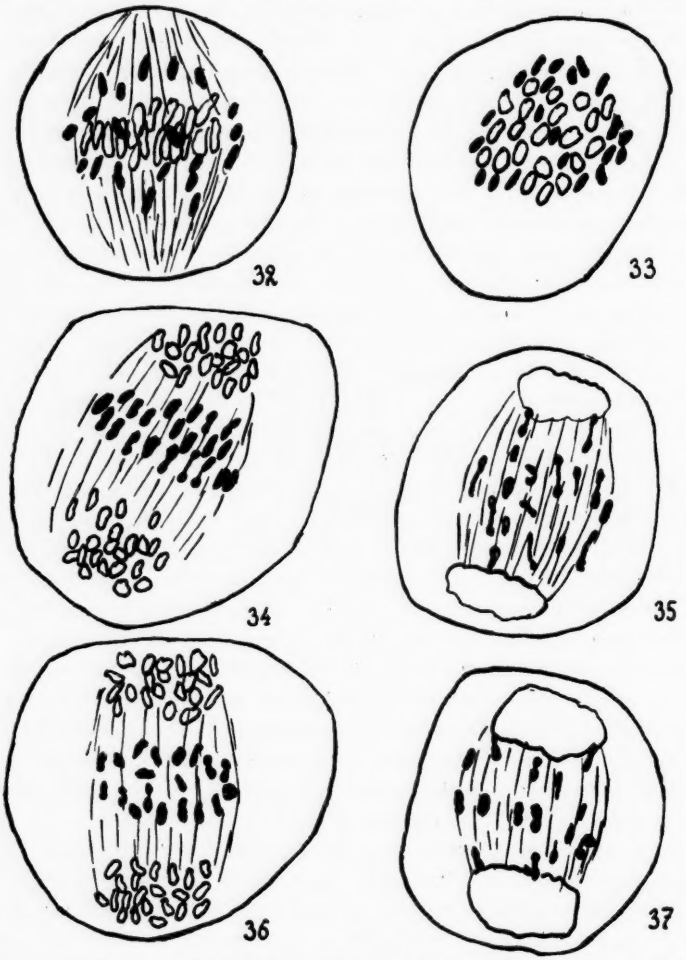
of the univalents counted at this stage in the ring were: 14 in 10 PMC's, 15 in 1 PMC, 16 in 1 PMC, and 18 in 2 PMC's.

At late anaphase the univalents begin to stretch. Here again this process is very irregular. At the same time as some univalents are stretched to many times their initial length the others remain unchanged, nearly spherical (Figs. 53—55). It also seems as if those univalents which were stretched earlier also begin to separate earlier into two halves, or, as happens more often, to move as a whole body to one of the poles. At the end of anaphase and the beginning of telophase the picture of the univalents is very irregular (Figs. 30, 31, 56). Some are already at the polar groups of the chromosomes, looking like two beads connected with 2 to 7 μ long chromosomal threads. Sometimes the size of two beads is very unequal and the picture resembles a fragmentation more than a division of the univalent. At the same time some of the univalents remain undivided on the equator, still others are much stretched in such a way that the two halves appear to be moving towards the opposite poles. Only few divide more or less regularly. Sometimes we can find a few univalents lying outside the spindle in the cytoplasm; they never show any stretching and they are surrounded by a sphere of more transparent cytoplasm. During diakinesis they are usually absorbed. For this peculiar stretching of the univalents their position on the spindle is no doubt necessary. It also seems that for their movement to the equator at metaphase and to the poles at anaphase they mostly use the peripheral part of the spindle. The number of the univalents going to each pole is quite at random, the most common case is when nearly half of the univalents are directed to each pole. The stretching and movement of the univalents are much delayed and are often prolonged till telophase. Many univalents are not included in either of the daughter nuclei. At interkinesis about 12 % of the PMC's show chromosomes lost in the cytoplasm.

The second division of meiosis is rather regular except that at II-M some chromosomes lie outside the plates and at II-A some chromosomes are lagging on the spindles. The picture of the tetrads is astonishingly regular. There are nearly 99 % poor pollen.

Hybrid No. 4 [*G. pyrenaicum* (42) $\times$ *G. rivale* (21)]. — In this hybrid the most common configuration is $21_{II} + 21_{I}$. The course of meiosis is very similar to the meiosis in hybrid No. 3 described above (Figs. 32—37, 16—26). It seems as though the rings of the univalents are less regular at anaphase. The picture of univalent stretching and movements is quite the same. Perhaps I have seen somewhat more uni-

valents that have really divided. As the univalents are here much more numerous, more of them rest unincluded in any nucleus at telophase.

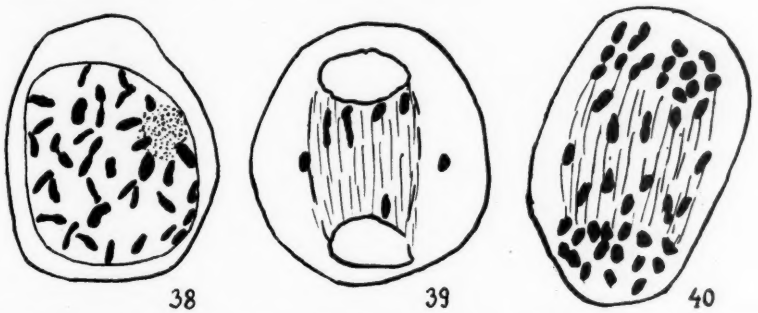


Figs. 32—37. Meiosis in hybrid No. 4. — Fig. 32. I-M with 21 univalents. — Fig. 33. I-M polar view. — Figs. 34, 36. I-A with univalents in the ring. — Fig. 35, 37. Late I-A with divided and stretched univalents.

In this hybrid inversion bridges are rather common. As a result of numerous delayed chromosomes, typical restitution nuclei were seen in two PMC's. In II-M numerous chromosomes lie outside the plates

(Fig. 27) and in II-A many chromosomes lag (Fig. 28). The pollen is nearly completely sterile.

Hybrid No. 5 [*G. macrophyllum* (21) × *G. rivale* (21)]. — This hybrid is asyndetic with only 42 univalents or more often with a few (1—7) bivalents. As in other hybrids, the univalents show pronounced secondary pairing and it is often difficult to distinguish a pair of univalents from a loose bivalent (Fig. 38). Notwithstanding this difficulty, it is quite certain that at diakinesis the number of bivalents is higher than at metaphase. Quite asynaptic cells are very rare at diakinesis. The picture of early metaphase is here very peculiar and is sometimes more like the anaphase in hybrids Nos. 3 and 4. The few bivalents (if

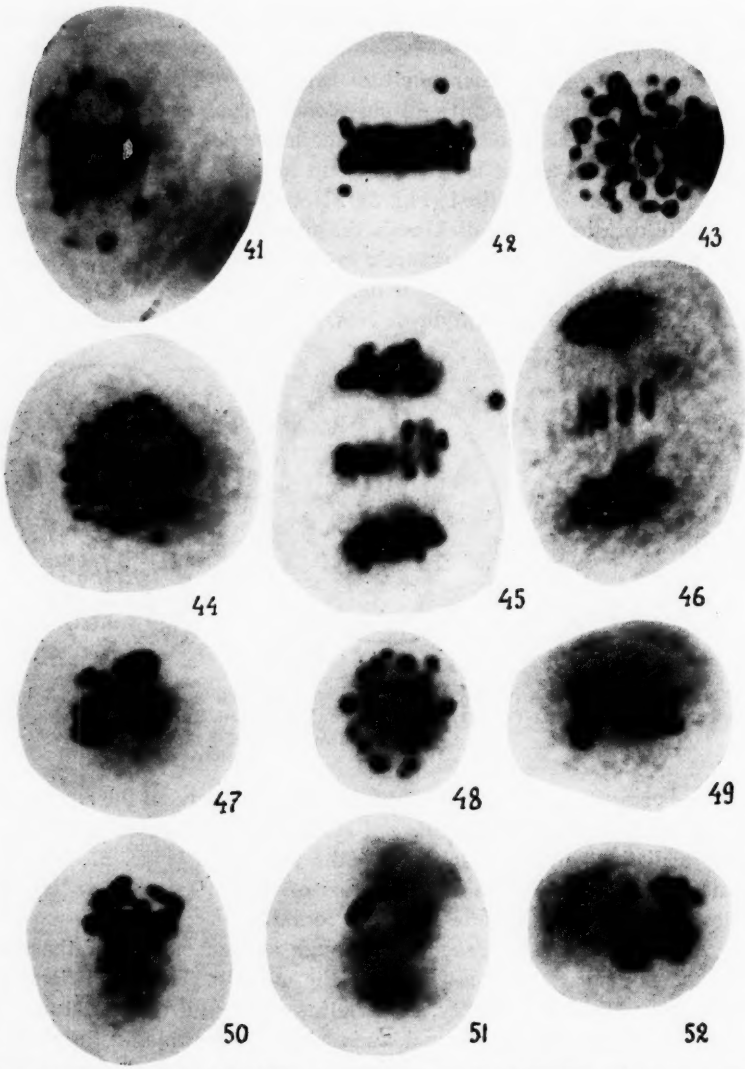


Figs. 38—40. Meiosis in hybrid No. 5. — Fig. 38. Diakinesis with 2 or 3 loose bivalents. — Fig. 39. I-T with undivided and stretched univalents. — Fig. 40. Probably I-A.

they are present at all) with some of the univalents are on the equatorial plane, the rest of the univalents are scattered on the spindle, but they show a distinct accumulation round the two poles (Figs. 57, 59, 63). Sometimes a picture with three groups of chromosomes: two on the poles and one in the middle are to be found.

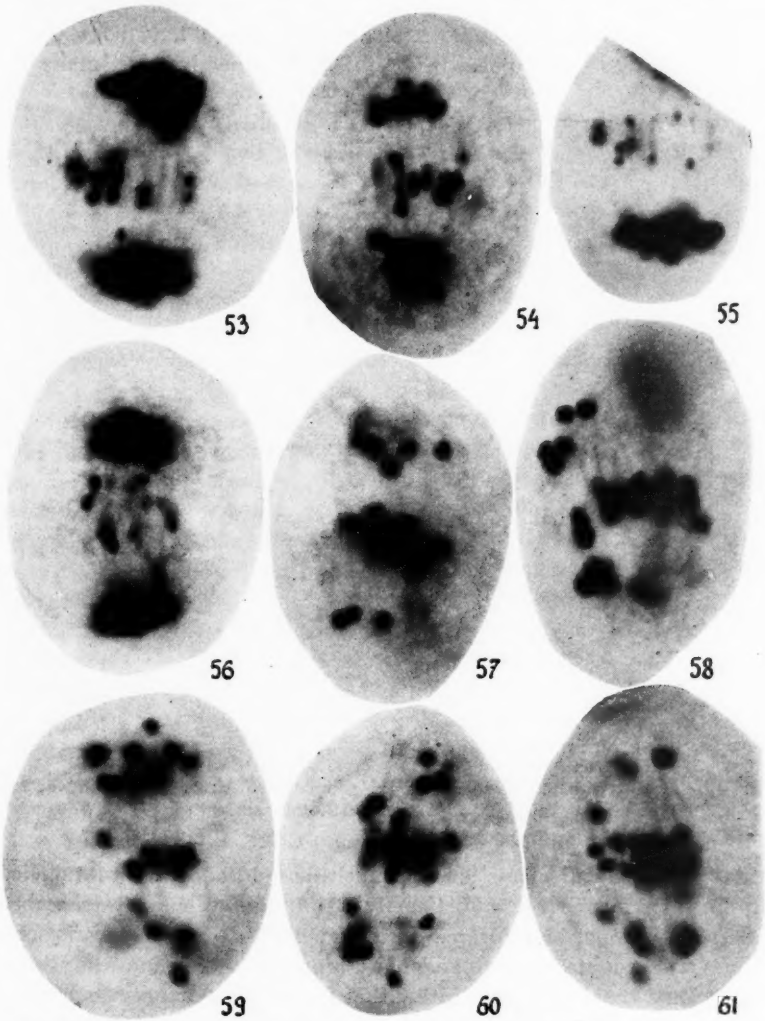
At late metaphase it seems as though at least a part of the univalents lying near the poles move to the equator. This is difficult to determine with full certainty, as seriation of the different pictures in a hybrid with such irregular meiosis is nearly impossible. In the pollen-sacs in *Geum* there is some regularity in the arrangement of PMC's according to the stage of meiosis.

At early anaphase, when the bivalents begin to divide the number of univalents lying at the poles is less than in the stage interpreted here as early metaphase (Figs. 58, 60—62, 64). When the bivalent halves



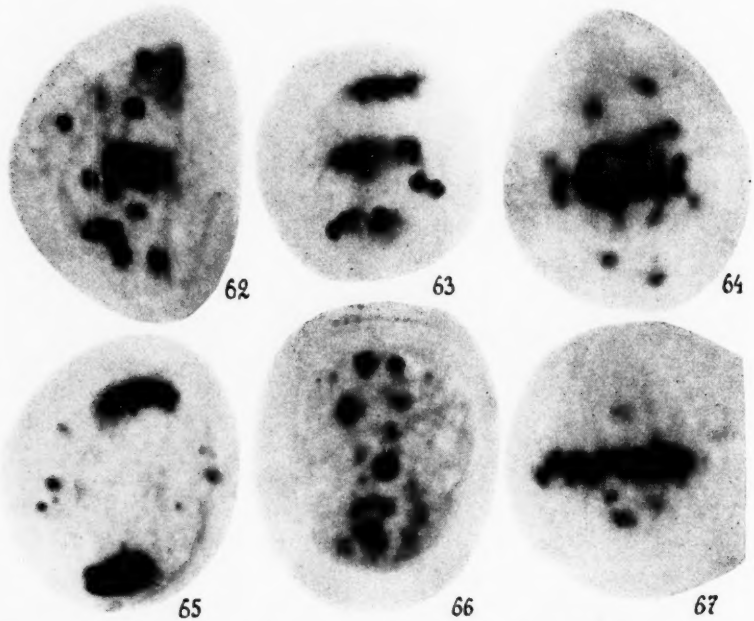
Figs. 41—52. Meiosis in hybrid No. 3. — Fig. 41. Earlier I-M with univalents on the spindle. — Fig. 42. Late I-M with nearly all univalents on the equatorial plane. — Figs. 43—44. I-M with univalents on the periphery of the bivalent plate. — Figs. 45—46. I-A with ring of univalents from side view. — Figs. 47—49 and 50—52 represent photographs from polar view of two cells at I-A at three levels with two anaphasal groups of bivalent halves (left and right) and the univalent ring (in the middle).

have reached the poles (where some univalents are already present) the rest of the univalents are scattered on the whole spindle (Fig. 40). Some of them may be just on the equator, but they never form such



Figs. 53—61. Meiosis in hybrid No. 4 (Figs. 53—56) and hybrid No. 5 (Figs. 57—61). — Figs. 53—56. Late I-A with divided and stretched univalents. — Figs. 57—61. I-M with different degrees of univalent grouping at the poles.

a regular ring or plate as in hybrids Nos. 3 and 4. Their subsequent behaviour seems to depend upon their position on the spindle. Those which were on the spindle move without any change of shape and much quicker and earlier to the nearer pole. Those that were on the equator show the same peculiar stretching and, after a long delay, move as whole bodies to the poles (Fig. 39). As in other hybrids, many of



Figs. 62—67. Meiosis in hybrid No. 5. — Figs. 62—64. Early I-M to early I-A. — Fig. 65. Late I-A with univalents out of the spindle. — Fig. 66. Restitution nucleus. — Fig. 67. II-M from restitution nucleus.

these stretched univalents are too much delayed and are not included in daughter nuclei (Fig. 65). Usually at interkinesis they are absorbed and no micronuclei are formed. In the few PMC's in which the chromosomes were counted in both anaphasal groups the total sum was always 42 or somewhat higher, indicating that no or only few univalents actually divided. It was unexpected that, notwithstanding the irregular course of meiosis, the equal distribution of 21 chromosomes in each anaphasal group was found many times. Some few restitution nuclei

were also observed (Figs. 66, 67). The second division is rather regular. The tetrads look regular but some dyads and tetrads with unequal microspores are to be found. The pollen is nearly completely sterile.

DISCUSSION.

As will have been noticed, the differences in the behaviour of univalents among five hybrids described here are very distinct. Whereas in hybrids Nos. 3 and 4 all univalents form a ring or plate on the equator, in hybrids Nos. 1, 2 and 5 the majority of them rest on the spindle. In hybrid No. 5 the univalents show a tendency at early metaphase to be grouped near the poles, which was not observed in any of the others.

We may ask, what are the causes of these differences in univalent behaviour? Two main factors could play the major rôle. Firstly, the differences in the genotypical constitution and, secondly, the different numerical relationships between uni- and bi-valents.

All hybrids studied here have one set of 21 *rivale* chromosomes in common. The other sets in hybrids Nos. 2, 3 and 4 consist of 14, 35 and 42 chromosomes that pair with the *rivale* set, leaving surplus chromosomes unpaired. In hybrids Nos. 1 and 5 all three species have the same chromosome number, 21, and their chromosomal sets are at least partially homologous. This is directly seen from the conjugation in hybrid No. 1 (with about 65 % of PMC's with 21_{II}). For the *macrophyllum* chromosomes this evidence could be obtained from its hybrid with *Geum canadense* having nearly the same type of conjugation as in hybrid No. 1. This indicates that the high degree of asynapsis in hybrid No. 5 is not due to the lack of chromosome homology (owing to structural or genic differences) but rather to the action of some gene (or genes) causing asynapsis (failure or reduction of chiasma formation, premature desynapsis).

I think that the genetical differences among the hybrids studied are probably more important for the degree of pairing at meiosis than for the univalent behaviour. It seems more probable that the different behaviour of the univalents is due rather to the different numerical relations among uni- and bi-valents.

From the comparison of meiosis in these hybrids the following conclusions could be drawn:

(1) The movement, congression, orientation, splitting and separation of the bivalents are quite normal in all hybrids.

(2) The timing of the bivalent cycle is also quite normal and independent of the univalent behaviour.

(3) It seems as though the whole cycle of the first meiotic division, the onset and end of every succeeding meiotical phase, is uninfluenced by the position and state of the univalents, even if they represent the majority of the chromosomes.

(4) The spindle formation and its elongation during anaphase is quite normal in all hybrids. It is always bipolar and symmetrical.

(5) The univalent behaviour during meiosis seems chiefly to depend on two factors: their capacity to move to the equator and their capacity for proper orientation and splitting.

(6) The question of the univalent movements is very obscure. Two kinds of movements must be discussed: in transverse and longitudinal directions (in relation to the spindle axis). For the longitudinal movements forces of attraction or repulsion between poles and centromeres are responsible. Sometimes an asymmetric structure of the spindle itself may be responsible, as in EMC's of *Caninae* roses (TÄCKHOLM, 1922), where all univalents move to the micropylar pole, whereas in PMC's with no asymmetry in the spindle they are distributed at random. It is also possible that the behaviour of the univalents resulting from desynapsis may be different from that of true univalents which have never paired in prophase. In hybrids Nos. 2, 3 and 4 most of the univalents are probably true ones and most of them never go to the poles at anaphase together with bivalents. In hybrids Nos. 1 and 5, where at least a part of the univalents result from desynapsis, some of them pass to the poles together with the bivalent halves, behaving like prematurely disjoined bivalents.

(7) Concerning the transverse movements of the univalents we know almost nothing. ÖSTERGREN (1945) suggests that some transverse equilibria on the spindle must exist. In hybrids Nos. 3 and 4, where the univalents form a peripheral ring, some kind of centrifugal force must operate, the nature of which is quite unknown. The rings could only be the result of the fact that the bivalents have earlier occupied the central part of the equatorial plane. It seems that for the movement of the univalents the relation between the diameter of the bivalent plate and the whole spindle on the equator might have some significance. When the plate of bivalents only occupies the central part of the equatorial plane, leaving the peripheral part free, the univalents can more easily move through peripheral parts of the spindle to form a ring round the bivalents, as in hybrids Nos. 3 and 4. The same behaviour

of the univalents has been described in many other hybrids, *e. g.*, in the *Triticum* and *Aegilops* group (KIHARA, 1930; AASE, 1930, and others), in such hybrids as *Aegilotriticum* (28) × *Ae. ovata* (14) with $14_{II} + 14_{I}$, *Aegilotriticum* (28) × *Tr. spelta* (21) with $14_{II} + 21_{I}$, *Ae. cylindrica* (14) × *Tr. vulgare* (21) with $7_{II} + 21_{I}$ and others, or in *Rosa* (TÄCKHOLM, 1922) in the group *Caninae* with configurations of $7_{II} + 14_{I}$, $7_{II} + 21_{I}$ and $7_{II} + 28_{I}$. In all these hybrids the plate of bivalents leaves the peripheral part of the spindle free.

(8) In hybrids Nos. 1 and 2 with relatively more numerous bivalents, which at metaphase form a large plate occupying nearly all the diameter of the spindle on the equator, the conditions for the univalent movement might be different. There is no peripheral part for the univalent movement and no conditions for a ring formation, and the univalents rest scattered on the spindle. In *Triticum*, too, this type of univalent behaviour is most typical of hybrids with a low number of univalents, as in pentaploid hybrids with $14_{II} + 7_{I}$. Of course, some transitional types occur between the arrangement with all univalents on the equator and all on the spindle, sometimes, even in one and the same hybrid.

(9) The movements and orientation of the univalents in asynaptic or nearly asynaptic hybrids like our hybrid No. 5 are most peculiar. KIHARA (1930) has described in *Tr. spelta* (21) × *Ae. triuncialis* (14), which is nearly asynaptic (but in other hybrids with more bivalents too), that the univalents at early metaphase are often grouped in the neighbourhood of the poles. Such figures are also drawn by TÄCKHOLM (1922) for roses. KIHARA (1930) supposes that some pictures in ROSENBERG's paper (1917) on the meiosis of asynaptic *Hieracium* of *laevigatum* type, which were described as anaphasic with two groups of univalents on the poles (semiheterotypic division, according to ROSENBERG), might in reality represent early metaphasic stages.

Perhaps, in some asyndetic hybrids the univalents are not true univalents but result from premature desynapsis and this could at least in part explain their peculiar behaviour. In our hybrid No. 5 some univalents move at metaphase to the equator, but they never form a regular plate. That most of them never reach the equator could be the result of unbalanced timing of univalent movements and telophasal and interkinetic changes in PMC's. KIHARA (1930) writes that if the regression (the transition to the stage of interphase) begins earlier, when the univalents are still on the poles, we obtain two nuclei (ROSENBERG's semiheterotypic division) if later, when the univalents are nearer the

equator, restitution nuclei might be formed. It seems that in general the univalents are more delayed in their movements in asynaptic hybrids than in hybrids with more bivalents. Perhaps the presence of the bivalents (and their influence on the spindle formation) is a stimulus to the movements and division of the univalents. In other asynaptic hybrids the formation of a regular plate of univalents and their regular division have been described. In *Hieracium* ROSENBERG (1917) states that in such cases (*e. g.*, *H. pseudoillyricum*) the univalents behave like and have even the shape of mitotic chromosomes and the whole division is like mitosis.

(10) In all hybrids the congression, division and movement to the poles of the univalents are always delayed as compared with bivalents. What is early anaphase for bivalents is still metaphase for univalents. The anaphase of the univalents usually corresponds to telophase in the daughter nuclei resulting from bivalent separation.

(11) At anaphase, when the bivalent halves are already on the poles the univalents in hybrids Nos. 3 and 4 always remain on the periphery of the equatorial plane, forming a ring and not moving centripetally. This may indicate that the central part of the spindle has properties different from the peripheral portion. If some kinds of elastic »traction fibres» exist, as some authors suppose, they could not exist in the central part of the spindle after the bivalent halves have gone to the poles but still do so in peripheral parts.

(12) The splitting of the univalents and their actual division into two parts are two separate processes. AASE (1930) writes (for *Triticinae* hybrids): »... If, however, the movement to the poles is delayed and the anaphasic split occurs, it affects simultaneously all the univalents wherever they lie on the spindle. A univalent lying at the equator will actually divide, adding a half to each pole. A dividing univalent lying off the equator will add both its halves to the nearer pole.» This signifies that at anaphase (at least in some hybrids) the univalents are split into chromatids without relation to their position on the spindle. In *Geum* material I was unable to determine the moment at which the univalents split. It seems very probable that in hybrids 1, 2 and 5 the majority of the univalents that lag on the spindle far off the equator are segregated to both daughter nuclei before they are split.

(13) The rare univalents in hybrids Nos. 1, 2 and 5 and all or the majority of univalents in hybrids Nos. 3 and 4 that lay on the equator or near it behave differently. Much delayed in comparison with bivalents they split and divide. In hybrids with regular division of univalents

KIHARA (1930) states that for normal division an exact orientation of univalents parallel to the equatorial plane is necessary. He has observed such orientation not only for univalents lying on the equator but also for those that were somewhat off the equator. For normal division the univalents must be split and orientated, which is usually done only in an equatorial position. I think that the mechanism for the orientation of a double univalent must be the same as for bivalents, resulting from the forces of attraction and repulsion between poles and polarized centromeres. Usually it may be noticed that for a proper orientation the univalents do not possess the same ability as the bivalents. Probably, this frequent misorientation of the univalents is not only the result of their inexact congression to the equator but is due to some other unknown factors. According to KIHARA (1930) and other cytogenetists, the univalents divide at times in a position off the equator. This is still more difficult to explain and was never observed in my *Geum* material. Usually the misorientated univalents do not divide at all or are misdivided by transverse division at the centromere (DARLINGTON, 1939; KOLLER, 1938).

(14) The peculiar differences in the behaviour of the univalents on the equator in hybrids Nos. 3 and 4 (and in less striking form in other hybrids described here), ranging from normal division through different degrees of stretching to no division at all, are probably due to different degrees in their orientation. Owing to the small size and nearly spherical shape of the univalents it was impossible to observe directly the kind of orientation they assumed but I think that different degrees of orientation must exist. The peculiar stretching of the univalents together with the movement of both parts to one pole must probably result from inexact orientation in relation to both poles. The univalent is stretched by the repulsion forces of two centromeres and is then attracted to that pole to which one of the centromeres is nearer or perhaps, more accurately, orientated. Of course, this is only a tentative hypothesis. It does not explain why some univalent halves going to opposite poles are united by a thin chromatic thread and why the parts of the stretched univalents are often of very dissimilar size. It is also possible that sometimes some kind of »stickiness» occurs, causing difficulties in the separation of the univalent parts and that, instead of a longitudinal split, sometimes a kind of transverse fragmentation due to stretching may happen. To say anything more decisive is not possible on account of lack of evidence.

In the literature describing meiosis with univalents I have found many descriptions or only pictures indicating similar univalent behaviour. It is interesting that most of these descriptions concerned genera related to *Geum* from the family Rosaceae. For instance, in the hybrid *Fragaria nipponica* (7) × *Fr. elatior* (21) F. A. LILIENFELD (1933) has found the configuration $14_{II} + 7_{I}$. The univalents are scattered on the spindle and distributed at random, undivided to the poles. But some of them behave otherwise, as LILIENFELD describes: »... Vereinzelt sieht man aber Univalente sich so verhalten, wie in Abb. 14. Hier sind drei verzögerte Univalente zu sehen, die wahrscheinlich in Längsteilung begriffen sind. Hin und wieder hat man in ähnlichen Fällen den Eindruck, als ob es sich nicht um Längsteilung, sondern um Quersegmentierung bezw. Fragmentierung handelte.» Fig. 14 and the description of LILIENFELD are very compatible with my observations in *Geum*. For the hybrid *Fr. chiloensis* (28) × *Fr. bracteata* (7) with $7_{II} + 21_{I}$ ICHIJIMA (1930) has given pictures of anaphase division of univalents (Nos. 51 and 54) very similar to those of our hybrids Nos. 3 and 4. Similar pictures were also published for *Potentilla* (POPOFF, 1939, Fig. 19; ARAKI, 1932, Fig. 9; A. and G. MÜNTZING, 1941, Figs. 29, 71; 1943, Fig. 9) and an oral communication made by Professor MÜNTZING also belongs here. For *Rosa* ERLANSSON (1929, Figs. 34 and 54) writes: »... Some of the univalents seem to have difficulties in dividing, so that the chromosomes become drawn out along the spindle fibres between the two poles.» Also in other families the univalents divide at times in a similar abnormal way. GATES (1915), for the trisomic mutant *laeta* of *Oe. Lamarckiana*, gives the following description of univalent division (p. 183): »... it is not a regular longitudinal split, but rather an irregular pulling apart transversely, leaving a trail of chromatin behind ...» Similar pictures are drawn for *Nicotiana* hybrids by CHRISTOFF (1928, Figs. 47 and 48).

Acknowledgements. — I wish to acknowledge my gratitude to the Director of the Institute of Genetics in Lund, Professor A. MÜNTZING, for the opportunity afforded me of carrying out these investigations in his laboratory, and to express my thanks to Mr. P. BERNSTÖM and Dr. A. LIMA DE FARIA for constant help and interest during my work at the Institute as well as to Mr. N. NYBOM for assistance in making the microphotographs.

LITERATURE CITED.

1. AASE, H. C. 1930. Cytology of *Triticum, Secale, Aegilops* hybrids with reference to phylogeny. — Research Studies of the State College of Washington, 2, No. 1, pp. 5—66.
2. ARAKI, S. 1932. Karyologische Untersuchungen über Artbastarde zwischen *Potentilla chinensis* (haploid 1*n*) und *P. nipponica* (haploid 2*n*). — Journ. of Sc. of the Hiroshima Univ. Ser. B, Div. 2, 1, pp. 103—116.
3. CHRISTOFF, M. 1928. Cytological studies in the genus *Nicotiana*. — Genetics, 13, pp. 233—255.
4. DARLINGTON, C. D. 1939. Misdivision and the genetics of the centromere. — Journ. of Gen., 37, pp. 341—364.
5. ERLANSSON, E. W. 1929. Cytological conditions and evidences for hybridity in North American wild roses. — The Bot. Gazette, 87, pp. 443—506.
6. GATES, R. R. 1915. The mutation factor in evolution with particular reference to *Oenothera*. — London.
7. ICHIJIMA, K. 1930. Studies on the genetics of *Fragaria*. — Ztschr. f. ind. Abst.- u. Vererb.-Lehre, 55, pp. 300—347.
8. KIHARA, H. 1930. Genomanalyse bei *Triticum* und *Aegilops*. II. — Cytologia, 2, pp. 106—155.
9. KOLLER, P. C. 1938. Asynapsis in *Pisum sativum*. — Journ. of Gen., 36, pp. 275—305.
10. LILIENFELD, F. A. 1933. Karyologische und genetische Studien in *Fragaria*. I. — Jap. Journ. of Bot., 6, pp. 425—458.
11. MÜNTZING, A. and G. 1943. Spontaneous changes in chromosome number in apomictic *Potentilla collina*. — Hereditas, XXIX, pp. 451—460.
12. — 1944. A pentaploid F_1 hybrid between two diploid *Potentilla* species. — Hereditas, XXX, pp. 631—638.
13. POPOFF, A. 1939. Karyologische Studien über die Entwicklungsvorgänge in der Gattung *Potentilla*. — Ztschr. d. Landwirtsch. Versuchsstat. in Bulgarien, 9, pp. 1—58.
14. ROSENBERG, O. 1917. Die Reduktionsteilung und ihre Degeneration in *Hieracium*. — Svensk Bot. Tidskr., 11, pp. 145—205.
15. TÄCKHOLM, G. 1922. Zytologische Studien über die Gattung *Rosa*. — Acta Horti Bergiani, 7, pp. 97—381.
16. ÖSTERGREN, G. 1945. Transverse equilibria on the spindle. — Bot. Notiser 1945, pp. 467—468.

ABSTRACTS — KURZE MITTEILUNGEN

TORSTEN WICKBOM: A new list of chromosome numbers in Anura.

During the last years Amphibians have become a rather popular material for experimental cytologic investigations. Unfortunately, however, a great many of the previous cytologic works on that group are very difficult to find as they were published in rather obscure periodicals. Earlier lists of the chromosome numbers in Vertebrata (OGUMA and MAKINO, 1937; MC CLUNG, 1939) are already antiquated.

The new list published here is based upon the taxonomic system of NOBLE (1931). The haploid chromosome number and the chromosome morphology are given for every species investigated. The morphology is marked by »V» = metacentric chromosome; »I» = acrocentric chromosome.

Since cytologically discernible sex chromosomes are lacking in Anura (WICKBOM, 1945), nothing is said in the list about sex chromosomes of any species.

Antiquated references with chromosome numbers shown by later investigators to be erroneous are not included in the present list. They are easily found in the literature list of the correcting author. Most of this literature is also found in OGUMA and MAKINO's (1937) list.

Species	n=	Morphology	Author
SUBORDER II. OPISTOCOELA			
FAMILY DISCOGLOSSIDAE			
Alytes obstetricans	18	6 V; 12 I	WICKBOM, 1949
Bombina bombina	12	All V	WICKBOM, 1949
Bombina orientalis	12	All V	SATO, 1939
Bombina variegata	12	All V	GALGANO, 1933; WICKBOM, 1949
Discoglossus pictus	14	10 V; 4 I	WICKBOM, 1949
FAMILY PIPIDAE			
Xenopus laevis	18	6 V; 12 I	WICKBOM, 1949
SUBORDER III. ANOMOCOELA			
FAMILY PELOBATIDAE			
Pelobates fuscus	13	All V	WICKBOM, 1945
SUBORDER IV. PROCOELA			
FAMILY BUFONIDAE			
Bufo americanus	11	All V	WITSCHI, 1933
Bufo arenarium	11	All V	SAEZ *et al.*, 1936
Bufo bufo	11	All V	STOHLER, 1928; IRIKI, 1929; MINOUCHI and IRIKI, 1929; TCHOU-SU, 1931; WICKBOM, 1945
Bufo calamita	11	All V	STOHLER, 1928; WICKBOM, 1945
Bufo canorus	11	All V	WITSCHI, 1933
Bufo fowleri	11	All V	WITSCHI, 1933

Bufo lentiginosus	11	All V	WITSCHI, 1933
Bufo quercinus	11	All V	WITSCHI, 1933
Bufo raddei	11	All V	SATO, 1936
Bufo regularis	11	All V	WICKBOM, 1949
Bufo sachalinens.s	11	All V	MAKINO, 1932 a
Bufo viridis	11	All V	BECCARI, 1926; STOHLER, 1928; GALGANO, 1933; WICKBOM, 1945
FAMILY HYLIDAE			
Acris crepitans	11	All V	BUSHNELL *et al.*, 1939
Acris gryllus	11	All V	BUSHNELL *et al.*, 1939
Hyla arborea	12	All V	IRIKI, 1930, 1932 b; GALGANO, 1933; WICKBOM, 1945
Hyla avivoca	12	All V	BUSHNELL *et al.*, 1939
Hyla cinerea	12	All V	BUSHNELL *et al.*, 1939
Hyla versicolor	12	All V	BUSHNELL *et al.*, 1939
SUBORDER V. DIPLASIOCOELA			
FAMILY RANIDAE			
Rana arvalis	11	All V	DÜRKEN, 1938
	12	All V	WICKBOM, 1945
	13	All V	CEI, 1946
Rana catesbiana	13	All V	SWINGLE, 1917
Rana dalmatina	13	All V	CEI, 1946; MATTHEY, 1947
Rana esculenta	13	All V	DALCQ, 1930; GALGANO, 1933; WICKBOM, 1945; CEI, 1946
Rana graeca	13	All V	CEI, 1946
Rana limnocharis	13	All V	SATO, 1934
Rana nigromaculata	13	All V	IRIKI, 1932 a; KAWAMURA, 1939
Rana palustris	13	All V	PARMENTER, 1933
Rana pipiens	13	All V	PARMENTER, 1933
Rana rugosa	13	All V	IRIKI, 1932 a
Rana temporaria	13	All V	WITSCHI, 1922, 1924; MAKINO, 1932 a; GALGANO, 1933; PROKOFIEWA, 1935; DÜRKEN, 1938; WICKBOM, 1945
FAMILY POLYPEDATIDAE			
Polypedates buergeri	13	All V	SATO, 1934
Rhacophorus schlegelii	13	All V	MAKINO, 1932 b
FAMILY BREVICIPITIDAE			
Cacopoides tornieri	14	All V	SATO, 1936

Institute of Genetics, University of Stockholm.

Literature.

1. BECCARI, N. 1926. Le nombre des chromosomes dans les cellules génitales de *Bufo viridis*. — Ann. Anat. Path. 3: 841.
2. BUSHNELL, R. J., BUSHNELL, E. P. and PARKER, M. V. 1939. A chromosome study

of five members of the family Hylidae. — Journ. Tennessee Acid. Sci. 14: 209—215 (Biol. Abstr. 1939, No. 10722).

3. Cei, G. 1946. Note citologiche sulle cellule germinali maschili de »*Rana graeca*», di »*Rana arvalis*» e di »*Rana dalmatina*». — Monitore Zool. Ital. 55: 10—27.
4. Dalcq, A. 1930. La formule chromosomiale chez la grenouille. — Ann. Soc. Roy. med. nat. Bruxelles 1930: 10—15.
5. Dürken, B. 1938. Über die Keimdrüsen und die Chromosomen der Artbastarde *Rana arvalis* Nilss. × *Rana fusca* Rös. — Zeitschr. f. ind. Abst.- u. Vererbungslehre 74: 331—353.
6. Galgano, M. 1933. Evolutione dei spermatociti di I ordine e cromosomi pseudosessuali in alcune specie di Anfibi. — Arch. Ital. Anat. Embr. 32: 171—200.
7. Iriki, S. 1929. On the chromosomes of *Bufo bufo japonicus*. — Zool. Mag. 41: 491.
8. — 1930. Studies on Amphibian chromosomes. I. On the chromosomes of *Hyla arborea japonica*. — M. C. S. Kyoto Imp. Univ. Ser. B. 5: 1—19.
9. — 1932 a. Studies on Amphibian chromosomes. IV. On the chromosomes of *Rana rugosa* and *Rana nigromaculata*. — Sci. Rep. Tokyo Bunr. Daig. Sec. B. 1: 61—72.
10. — 1932 b. Studies on Amphibian chromosomes. V. Explanation of the sex chromosome type of *Hyla arborea japonica* from the standpoint of spiral structure. — Sci. Rep. Tokyo Bunr. Daig. Sec. B. 1: 73—80.
11. Kawamura, T. 1939. Artificial parthenogenesis in the frog. I. Chromosome numbers and their relation to the cleavage histories. — Journ. of Sci. Hiroshima Univ. Ser. B. Div., 1, 6: 115—218.
12. Makino, S. 1932 a. Notes on the chromosomes of *Rana temporaria* and *Bufo sachalinensis*. — Proc. Imp. Acad. Tokyo, 8: 23—24.
13. — 1932 b. Notes on the chromosomes of *Rhacophorus schlegelii schlegelii*. — Proc. Imp. Acad. Tokyo, 8: 24—26.
14. Matthey, R. 1947. Quelques formules chromosomiales. — Sci. Genetica III: 23—32.
15. Mc Clung, C. E. 1940. Chromosome numbers in animals. — Tabul. Biol. 18: 83—113.
16. Minouchi, O. and Iriki, S. 1931. Studies on Amphibian chromosomes. II. The chromosomes of *Bufo bufo japonicus*. — M. C. S. Kyoto Imp. Univ. Ser. B. 6: 39—43.
17. Oguma, K. and Makino, S. 1937. A new list of the chromosome numbers in Vertebrata (March 1937). — Journ. Fac. Sci. Imp. Univ. Sapporo, Ser. 6, 5: 297—356.
18. Parmenter, C. L. 1933. Haploid, diploid, triploid, and tetraploid chromosome numbers and their origin in parthenogenetically developed larvae and frogs of *Rana pipiens* and *Rana palustris*. — Journ. exp. Zool. 66: 409—453.
19. Prokofiewa, A. 1935. On the chromosome morphology of certain Amphibia. — Cytologia 6: 148—164.
20. Saez, F. A., Rojas, P., and de Robertis, E. 1936. Untersuchungen über die Geschlechtszellen der Amphibien (Anuren). I. Der meiotische Prozess bei *Bufo arenarium*. — Zeitschr. f. Zellforsch. u. mikr. Anat. 24: 727—777.
21. Sato, I. 1934. On the chromosomes of *Rana limnocharis* and *Polypedates buergeri*. — Zool. Mag. 46: 98—104.

22. SATO, I. 1936. Notes on the chromosomes of *Cacopoides tornieri* and *Bufo raddei*. — Zool. Mag. 48: 958—960.
23. — 1939. On the chromosomes of the Oriental bell-toad *Bombina orientalis*, with special reference to the multiple ring tetrad. — Journ. of Sci. Hiroshima Univ. Ser. B, 6: 53—70.
24. STOHLER, R. 1928. Cytologische Untersuchungen an den Keimdrüsen der mitteleuropäischen Kröten (*Bufo viridis* LAUR., *Bufo calamita* LAUR. und *Bufo vulgaris* LAUR.). — Zeitschr. f. Zellforsch. u. mikr. Anat. 7: 400—475.
25. SWINGLE, W. W. 1917. The accessory chromosome in a frog possessing hermaphroditic tendencies. — Biol. Bull. 33: 70—79.
26. TCHU-SU. 1931. Étude cytologique sur l'hybridation chez les Anoures. — Arch. Anat. Micr. 27: 1—105.
27. WICKBOM, T. 1945. Cytological studies on Dipnoi, Urodela, Anura, and *Emys*. — Hereditas XXXI: 241—346.
28. — 1949. Further cytological studies on Anura and Urodela. — Hereditas XXXV: 33—48.
29. WITSCHI, E. 1922. Chromosomen und Geschlecht bei *Rana temporaria*. — Zeitschr. f. ind. Abst.- u. Vererbungslehre 27: 253—255.
30. — 1924. Die Entwicklung der Keimzellen der *Rana temporaria*. I. Urkeimzellen und Spermatogenese. — Zeitschr. f. Zellen- u. Gewebelehre I: 523—561.
31. — 1933. Contribution to the cytology of Amphibian germ cells. I. Chromosomes in the spermatocyte divisions of five north American species of toads. — Cytologia 4: 171—181.

TORSTEN WICKBOM: The time factor of chromosome spiralization.

In his investigation on the chromosomes of Salmonidae, SVÄRDSON (1945, pp. 15—32) showed that the shortening of the mitotic chromosomes by spiralization from early metaphase to anaphase was not uniform in all chromosomes or chromosome arms. Instead there was a positive correlation between the length of arms and degree of shortening.

SVÄRDSON tried to explain this phenomenon by assuming a »time factor» of spiralization. If the spiralization starts at one or some points on the chromosome and »migrates» forward, a shorter chromosome or arm should have a lead in spiralization in middle or late prophase; a lead which could be made good only gradually at the end of the spiralization phase, by the longer chromosome or arm. »In that case the shorter chromosome arm during the prophase should shorten *more* and during the metaphase *less* than a long arm ... A greater shortening of the smaller arm in prophase, however, cannot be shown in my material, as the chromosomes then cannot be closely analyzed.» (SVÄRDSON, *l. c.*, pp. 29—30.)

These findings and the explanation of them strongly conflict with the common views about the stability of the »chromosome index» (see SVÄRDSON, *l. c.*, p. 26 for discussion).

Unfortunately, SVÄRDSON's arguments are somewhat weakened by the

fact that his material did not enable him to observe the earlier phases of the spiralization, *i. e.* the phase where the small chromosomes according to him should be most spiralized.

In the Amphibian material of the present author, however, some nuclei are found which may be used to demonstrate the whole spiralization from earliest prophase to late metaphase and thus complete SVÄRDSON's work. They clearly show that the chromosomes of Anurans and Urodelians behave as those of Salmonidae. No difference is found between Anurans and Urodelians in this respect.

Naturally, easily analyzable nuclei in prophase must be extremely rare. Faced with the choice between working with means from a large number of nuclei, most of which must have some more or less uncertain points, and working with a lower number, the interpretation of which is completely sure, the present author has decided to base his investigation upon the second alternative.

TABLE 1. *Chromosome length in early prophase and full metaphase. Bufo bufo.*

Pair No.	Length in prophase		Length in metaphase		Difference		
	Microns	% of set	Microns	% of set	Microns	% of prophase	Variation from mean
1	68	17,7	17	16,8	51	72,1	— 0,7
2	63	16,5	16	15,8	47	74,5	+ 1,7
3	50	13,1	14	13,8	36	72,0	— 0,8
4	48	12,6	13	12,8	35	73,0	+ 0,2
5	40	10,5	11	10,8	29	72,5	— 0,3
6	30	7,9	8	7,9	22	73,3	+ 0,5
7	23	6,0	6	5,9	17	73,9	+ 1,1
8	18	4,7	5	4,9	13	72,2	— 0,6
9	15	3,9	4	3,9	11	73,3	+ 0,5
10	14	3,7	4	3,9	10	71,4	— 1,4
11	13	3,4	3,5	3,5	9,5	73,0	+ 0,2
Total	382	100,0	101,5	100,0	280,5	72,8	—

The material consists of one early prophase of *Bufo bufo* compared with a good metaphase of the same individual (Table 1), one middle prophase and one metaphase from a single individual of *Salamandra salamandra* (Table 2) and one middle prophase and one metaphase from one single individual of *Hyla arborea* (Table 3). All nuclei are from early spermatogonial cells. The metaphases were compared with some other good metaphases from the same species and were found to be thoroughly typical.

The chromosomes were drawn with the aid of a camera lucida at a magnification of 5000 ×. The drawings were measured and the chromosomes placed together in pairs. As appears from the description of the chromosomes of these species (WICKBOM, 1945, 1949) all chromosomes of these species are almost mediocentric. After having been handled with acetocarmin, the centro-

TABLE 2. *Chromosome length in mid prophase and full metaphase. Salamandra salamandra.*

Pair No.	Length in prophase		Length in metaphase		Difference		
	Microns	% of set	Microns	% of set	Microns	% of prophase	Variation from mean
1	41	14,0	20	12,3	21	51,2	+ 8,1
2	37	12,6	19	11,8	18	48,7	+ 5,6
3	33	11,3	18	11,2	15	45,5	+ 2,4
4	31	10,6	17	10,6	14	45,2	+ 2,1
5	27	9,2	15	9,3	12	44,4	+ 1,3
6	26	8,9	14	8,7	12	46,1	+ 3,0
7	23	7,9	13	8,1	10	43,5	+ 0,4
8	21	7,2	12	7,5	9	42,9	— 0,2
9	16	5,5	10	6,2	6	37,5	— 5,6
10	15	5,2	9	5,6	6	40,0	— 3,1
11	13	4,5	8	5,0	5	38,5	— 4,6
12	9	3,1	6	3,7	3	33,3	— 9,8
Total	292	100,0	161	100,0	131	43,1	—

TABLE 3. *Chromosome length in mid prophase and full metaphase. Hyla arborea.*

Pair No.	Length in prophase		Length in metaphase		Difference		
	Microns	% of set	Microns	% of set	Microns	% of prophase	Variation from mean
1	32	23,4	12	15,8	20	62,5	+ 25,8
2	22	16,1	10	13,1	12	54,5	+ 17,8
3	18	13,1	10	13,1	8	44,4	+ 7,7
4	13	9,5	8	10,5	5	38,5	+ 1,8
5	12	8,8	8	10,5	4	33,3	— 3,4
6	8	5,8	6	7,8	2	25,0	— 11,7
7	7	5,1	5	6,6	2	28,6	— 8,1
8	6	4,4	4	5,3	2	33,3	— 3,4
9	6	4,4	4	5,3	2	33,3	— 3,4
10	5	3,6	3,5	4,6	1,5	30,0	— 6,7
11	5	3,6	3,0	3,9	2	40,0	+ 3,3
12	3	2,2	2,5	3,3	0,5	16,7	— 20,0
Total	137	100,0	76,0	100,0	61,0	36,7	—

meres of these species are not always distinguishable in prophase. Therefore it is important that all chromosomes in the set are uniform as regards the arm index, because otherwise the facts pointed out by SVÄRDSON (*l. c.*) would make the analysis impossible.

The results are summarized in Tables 1—3.

From the tables the following conclusions are drawn:

(1) Between early prophase and metaphase all chromosomes are shortened by the same percentage irrespective of length (Table 1).

(2) Between mid prophase and metaphase the shortening is proportionally much greater in the long chromosomes than in the small ones (Tables 2 and 3).

(3) Thus between early prophase and mid prophase the opposite must have taken place. The shortening must have been proportionally stronger in the small chromosomes than in the long ones.

This is in accordance with SVÄRDSON's findings and theories.

As regards the cause of this phenomenon, the present author shares SVÄRDSON's opinion that the spiralization, whatever its physical origin may be, migrates along the chromosome arm so slowly that *there is a time factor in spiralization.*

Institute of Genetics, University of Stockholm.

Literature cited.

1. SVÄRDSON, G. 1945. Chromosome studies on Salmonidae. — Medd. fr. Stat. Unders. o. Försöksanst. f. Sötvatt.-fisk. 23: 1—151.
2. WICKBOM, T. 1945. Cytological studies on Dipnoi, Urodela, Anura, and *Emys*. — Hereditas XXXI: 241—346.
3. — 1949. Further cytological studies on Anura and Urodela. — Hereditas XXXV: 33—48.

GUDMUND SMITH: A new methodical aspect in psychological genetics.

The basic question of this thesis is: How do negative after-images and eidetic images look when seen from a hereditary psychological angle? Since the research has a psychological bias, however, we intend to place our results in relation to a person and not to exclude the latter for the benefit of general laws. Thus our description must not be confined to the behaviour in the perceptual experiments but rather extended to the person as a whole.

First we define our terms:

Surroundings: the objective constellation of stimuli around the individual (function).

Relevant region: the constellation of stimuli »chosen» and given »Gestalt» by the individual (function). Thus representing in terms of surroundings the relation individual (function) surroundings.

Heredity: psychologically a basic regulating principle (limes) within the individual (function). Often the terms »differences» and »non-differences» in heredity are the only ones used.

Individual (function) and surroundings (not relevant region) converge in the behaviour. The development of the individual (function) can be *peristatico-stable* or *peristatico-unstable*; these terms have always reference to heredity; *i. e.* an aim from the beginning of the development. A p-stable heredity (disposition) lets the development preserve its »profile» when the surroundings vary; a p-unstable heredity (disposition) allows changing development with variation of the surroundings. Twin research is a path for determining

p-stability and p-instability. The twin methods, however, must be used with much more caution than has been customary.

The twin population was limited to Lund and the surrounding country. The similarity diagnosis was performed according to ESSEN-MÖLLER's (1941) method. Altogether there were 13 male IT (pairs), 19 female IT, 16 male FT and 13 female FT, 8 to 69 years old.

The investigation of the personality discordance within the twin pairs and of the pair-relations among other things gave the following results: (1) The personality differences are much less within IT pairs than within FT pairs. (2) Each IT pair shows in this cross-section of time a discordance peculiar to the pair. (3) This discordance may increase or decrease under influence of differences in surroundings. But in its quality it seems to remain about the same. (4) The more highly developed twins have not only better conditions for analysing the differences between themselves but obviously also by their more extensive »choice-of-fields» greater possibilities for stressing a difference. (5) Within IT co-operation takes the form of a parallel arrangement, within FT more the form of superior and inferior arrangement or of no co-operation at all. In reality the »roles» vary from pair to pair because each pair has its own ground structure and discordance.

The after-image and eidetic image experiments were performed with a new apparatus. We tested the ability to call forth eidetic images, the duration and periodicity of after-images and the size of projected after-images at different distances. Both ordinary and intermittent visualization were used. The peculiarities of the after-image and eidetic image could be better understood than before in terms of relevant region and surroundings. Only results with reference to genetics are summarized here.

The intra-class correlation between eidetic indices (signifying the eidetic ability) is positive and significant within IT pairs, not significant within FT pairs. The greatest difference in correlation is obtained when using the differences between indices without and with intermittence. Differences in eidetic indices, within IT as well as FT, are often associated with differences in personality structure.

The intra-class correlation coefficients as regards after-image duration without or with intermittence are small. But when we compare the differences in reaction without and with intermittence we obtain a significant positive correlation within IT, no correlation within FT. Thus, from the viewpoint of a development this after-image function is peristatico-stable. Differences in this p-stable development are connected with differences in personality type.

The results from measuring the after-image size show that developments have occurred within the experiment. The basic values show positive correlation, both in IT and FT. In a preliminary experiment, however, only IT were correlated. This result may be ascribed to the subjects being able to choose surroundings relatively freely; and then IT chose more similar surroundings than FT. The positive correlation coefficients in the main experiments are caused by age and sex, *i. e.* certain resemblances of »inner» surroundings common to both IT and FT.

By an intra-pair computation of the correlation between the measurement

differences we obtain differences between IT and FT at distances where we found developmental tendencies: IT correlate but FT do not. As soon as we study the after-image function as a development we thus obtain peristatico-stability. A casuistic investigation seems to show that there is a connection between discordant after-image development and personality discordance. In its peristatico-stable reactions the after-image function has thus an »inner» relevant region which is only to a slight degree dependent on the outer surroundings. The experiment as a whole is, moreover, closely associated with the personality type.

The correlation between the »errors» without and with intermittence changes from IT to FT, from female twins to male, *etc.* This may be interpreted as differences in set against the experiments. A comparison between inter-pair differences in IT and FT seems to verify the hypothesis that adult (female) IT are more reserved than other twins towards the experiment. Further comparisons between IT and FT emphasize that dissimilarities of pair-situation play a role in the experimental results.

The differences *between* the twins are positively correlated to the differences *within* them.

Literature cited.

1. Essen-Möller, E. 1941. Empirische Ähnlichkeitsdiagnose bei Zwillingen. — Hereditas XXVII, pp. 1—50.
2. Smith, G. 1949. Psychological studies in twin differences. With reference to after-image and eidetic phenomena as well as more general personality characteristics. — Studia Psychologica et Paedagogica. Series altera. Investigationes 3.

Eeva Therman-Suomalainen: The action of pyrogallol on secondary constrictions.

In the course of the extensive studies of Levan and his coworkers concerning the action of inorganic as well as organic compounds on the structure and behaviour of the chromosomes a series of different phenols have also been tried in regard to their activity (Levan and Tjio, 1948 a and b). Most of the phenols used had a conspicuous fragmenting effect on the root-tip chromosomes of *Allium cepa*, pyrogallol being the most active agent in this respect.

The study on the structure of secondary constrictions in *Polygonatum* and *Smilacina* (Therman-Suomalainen, 1948, 1949) has been continued in a number of other plants having the same type of long, clear-cut constrictions which appear as colourless or faintly stained hiata in the chromosomes. In view of the striking results obtained by Levan and Tjio (*l. c.*) it was thought to be of interest to try what effect pyrogallol would have on this type of secondary constrictions. Of the plant species subjected to this treatment the following yielded the most conspicuous results: *Podophyllum emodi*, *Pisum arvense* and *Vicia sativa*. In all these it was observed that *the chromosomes were broken by the pyrogallol treatment at the secondary constrictions and only at these*. No such effects as abundant fragmentation or »mottling» of the

chromosomes, which were so common in *Allium*, could be detected in the present material. The fragmentation at the secondary constrictions was most pronounced at the mitotic prophases, at least with the method now used (4 hours in 0,000,5 mol/l or 0,001 mol/l pyrogallol). In the metaphase plates the chromosomes appeared contracted as after colchicine treatment and no fragmentation could be observed. At anaphase again fragmentation was seen in a few cells, which phenomenon was, however, very rare at this stage.

The present observation that the chromosomes break at the secondary constrictions might explain also the finding of LEVAN and TJIO (1948 b, p. 480): »The very morphology of the fragmentations indicates that an attack is made towards certain points of the chromosome». It might be thought that in this case, too, the chromosomes most easily would be broken at the secondary constrictions, the result being, however, obscured by the great susceptibility of *Allium* chromosomes to treatment with various chemicals which leads to breaks at other points too.

On the basis of the data described above it is suggested tentatively that pyrogallol in the first place attacks the chromosomes at the hiata created by the secondary constrictions.

The study on the secondary constrictions and their reactions is being continued in that the chromosomes of a number of plant species, belonging to different taxonomic groups, are subjected to treatment with different reagents.

State Horticultural Institute, Piikkiö, Finland, November, 1948.

Literature cited.

1. LEVAN, A. and TJIO, J. H. 1948 a. Chromosome fragmentation induced by phenols. — Hereditas XXXIV: 250—252.
2. — and — 1948 b. Induction of chromosome fragmentation by phenols. — Hereditas XXXIV: 453—484.
3. THERMAN-SUOMALAINEN, EEVA. 1948. The structure of secondary constrictions. — Hereditas XXXIV: 513—514.
4. — 1949. Investigations on secondary constrictions in *Polygonatum*. — Hereditas XXXV: 86—108.

ANTERO VAARAMA: The chromosome number of *Fumaria officinalis*.

In my earlier paper (VAARAMA, 1943) I have given the chromosome number $2n = 32$ for a few specimens of *Fumaria officinalis* collected in Finland. This number deviates both from the number ($2n = 14$) counted by SUGIURA (1940) in Japanese material and the number ($2n = 28$) found by WULFF (1937) in Schleswig-Holstein. The question therefore arose whether this number was a sporadical chromosome aberration or whether it represented an intraspecific chromosomal race with a wider distribution. To this purpose I have collected material from the countries surrounding the Baltic. In this task I have received kind support from the following colleagues: Dr. TYGE W. BÖCHER, Copenhagen; Dr. ASKELL LÖVE, Reykjavik; Dr. ANTON LANG, Tübingen; and Mr. R. ALAVA, Turku. The chromosome number has been

determined in several specimens from each locality. The material has been fixed with the craf-solution and stained with crystal violet. The following table contains the results of these counts:

Locality	n	$2n$
U. S. S. R., East-Carelia, Kenjärvi	—	32
» » Salmi, Käsnäselkä	16	—
Finland, Tyrväntö, Lepaa	16	32
» Piikkiö, Yltöinen	16	—
Sweden, Scania, Lund	—	32
» » Torrlösa	16	—
Denmark, Sjælland, Vindinge	—	32
» » Stoerkende	—	32
Germany, Berlin, Potsdam-Rehbrücke	16	—
» » Potsdam-Drewitz	16	—
» » Werder/Havel	16	32

The chromosome number is thus without exception $2n = 32$. It seems obvious that at least in Northern Europe and Northern Germany the form with 32 chromosomes is prevailing. *F. officinalis* with 28 chromosomes recorded by WULFF is evidently rare in this area. Whether this form with 28 chromosomes has a wider distribution and whether the type with 14 chromosomes is restricted to Japan remains to be decided on the basis of larger material.

NEGODI (1936, 1944) has shown that the most common basic number in the genus *Fumaria* is 7. In the related genera *Dicentra*, *Corydalis* and *Adlumia* (DARLINGTON and JANAKI AMMAL, 1945; NEGODI, 1944) the basic number 8 is prevailing. It seems probable that the form of *F. officinalis* with 32 chromosomes has originated from the type with 28 chromosomes owing to disturbances in the meiotic mechanism. The occurrence of such disturbances is doubtless favoured by the non-terminalized chiasmata which are commonly found in the meiosis of *F. officinalis*.

State Horticultural Institute, Piikkiö, Finland.

Literature cited.

1. DARLINGTON, C. D. and JANAKI AMMAL, E. K. 1945. Chromosome atlas of cultivated plants. — London.
2. NEGODI, G. 1936. Cariologia e filogenesi nel Genere *Fumaria* (Papaveraceae). — Riv. Biol. 20: 224—236.
3. — 1944. Contributo alla cariologia delle Papaveraceae, Subfam. Fumarioideae, con particolare riguardo ai Generi *Dicentra*, *Corydalis*, *Cisticapnos* e *Adlumia*. — Scientia Genetica 2: 1—25.
4. SUGIURA, T. 1940. Chromosome studies on Papaveraceae with special reference to the phylogeny. — Cytologia 10: 558—576.
5. VAARAMA, A. 1943. Beobachtungen über die Meiose bei einigen Anthropochoren. — Hereditas XXIX: 191—193.
6. WULFF, H. D. 1937. Chromosomenstudien an der schleswig-holsteinischen Angiospermen-Flora. I. — Ber. Deutsch. Bot. Ges. 55: 262—269.